09/817003
AA #6

62. Document ID: WO 200120015 A1

L9: Entry 62 of 63

File: DWPI

Mar 22, 2001

DERWENT-ACC-NO: 2001-257886
DERWENT-WEEK: 200148

TITLE: Introducing DNA into eukaryotic cells, by reverse transcription, used to produce arrays of reverse transfected cells, for use in high throughput screening, involves depositing DNA on a surface

PRIORITY-DATA: 2000US-0193580 (March 30, 2000), 1999US-0154737 (September 17, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 200120015 A1	March 22, 2001	E	040	C12N015/88

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
WO 200120015A1	September 18, 2000	2000WO-US25457	

INT-CL (IPC): C12N 15/10; C12N 15/88; C12Q 1/68

IN: SABATINI, D M

AB: NOVELTY - Introducing DNA into eukaryotic cells by reverse-transfection, comprising depositing a DNA mixture containing the DNA and a carrier protein, onto a surface in discrete, defined locations, and allowing it to dry, is new. The cells are then plated onto the surface, so that the DNA enters them., DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:, (1) introducing DNA into a eukaryotic cell, comprising:, (a) depositing a carrier protein-DNA mixture onto a surface in discrete, defined locations, and allowing it to dry;, (b) covering the surface with a lipid-based transfection reagent and maintaining the resulting product under conditions so that the DNA and transfection reagent form a complex;, (c) removing the reagent, to produce a surface bearing DNA; and, (d) plating the cells onto the surface, so that the DNA enters the cells;, (2) introducing DNA into eukaryotic cells, comprising:, (a) depositing a gelatin-DNA mixture onto a surface in discrete, defined locations, and allowing the mixture to dry;, (b) covering the surface with a lipid-based transfection reagent and maintaining the resulting product under conditions so that the DNA and transfection reagent form a complex;, (c) removing the reagent, to produce a surface bearing DNA; and, (d) plating the cells onto the surface, so that the DNA enters the cells;, (3) introducing DNA into eukaryotic cells, comprising:, (a) depositing a lipid-DNA mixture onto a surface in discrete, defined locations, the mixture containing DNA, a carrier protein, a sugar, a buffer facilitating DNA condensation, and a lipid-based transfection reagent, the mixture is allowed to dry; and, (b) plating the cells onto the surface bearing the lipid-DNA mixture, so that the DNA enters the cells;, (4) affixing DNA to a surface to produce an array of DNA in discrete, defined locations of known sequence or source, comprising spotting, the DNA mixture of the novelty, or of (1), (2), or (3), onto the surface, and allowing to dry, so that the DNA-containing spots remain affixed to the surface;, (5) producing an array on a surface of reverse transfected cells that contain defined DNA, comprising:, (a) spotting a gelatin-DNA mixture onto a surface in discrete, defined locations, and allowing to dry;, (b) covering the surface with a lipid-based transfection reagent and maintaining the resulting product under complex forming conditions;, (c) removing transfection reagent, producing a surface bearing DNA;, (d) adding cells to the surface; and, (e) maintaining the surface so that the DNA enters the cells;, (6) producing on a surface, an array of reverse transfected cells that contain defined DNA, comprising:, (a) spotting a lipid-DNA mixture onto the surface in discrete, defined locations, to produce spots and allowing it to dry; and, (b) plating cells on top of the surface produced in (a), and maintaining the surface, which contains dried lipid-DNA mixture cells to be reverse transfected, under conditions for cell growth and DNA entry into the cells;, (7) an array produced by the method of (4), (5), or (6)., USE - For introducing DNA into cells, and for producing arrays, of reverse transfected cells (claimed), used in high throughput screening assays.

L9: Entry 62 of 63

File: DWPI

Mar 22, 2001

DERWENT-ACC-NO: 2001-257886
DERWENT-WEEK: 200148
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TITLE: Introducing DNA into eukaryotic cells, by reverse transcription, used to produce arrays of reverse transfected cells, for use in high throughput screening, involves depositing DNA on a surface

AB TX:
(5) producing an array on a surface of reverse transfected cells that contain defined DNA, comprising:

ABTX:
(6) producing on a surface, an array of reverse transfected cells that contain defined DNA, comprising:

ABTX:
USE - For introducing DNA into cells, and for producing arrays, of reverse transfected cells (claimed), used in high throughput screening assays.

TTX:
INTRODUCING DNA EUKARYOTIC CELL REVERSE TRANSCRIBING PRODUCE ARRAY REVERSE TRANSFECTED CELL HIGH THROUGHPUT SCREEN DEPOSIT DNA SURFACE

63. Document ID: AU 9935727 A, WO 9955886 A1

L9: Entry 63 of 63 File: DWPI Nov 16, 1999

DERWENT-ACC-NO: 2000-086386
DERWENT-WEEK: 200015

TITLE: New function-based gene discovery method, useful to identify genes for gene therapy

PRIORITY-DATA: 1998US-0065775 (April 24, 1998)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 9935727 A	November 16, 1999		000	C12N015/64
WO 9955886 A1	November 4, 1999	E	068	C12N015/64

APPLICATION-DATA:

PUB-NO	APPL-DATE	APPL-NO	DESCRIPTOR
AU 9935727 A	April 21, 1999	1999AU-0035727	
AU 9935727A		WO 9955886	Based on
WO 9955886A1	April 21, 1999	1999WO-US08823	

INT-CL (IPC): C12N 15/64; C12N 15/66; C12Q 1/02; C12Q 1/68

IN: CEN, H, SUN, S

AB: NOVELTY - A new function-based gene discovery method uses a biological readout assay in which a 'bar-coded' cDNA library, i.e. a library contained in vectors comprising a unique oligonucleotide sequence ('bar-code') for each library clone, is screened., DETAILED DESCRIPTION - Biological readout assays comprise:, (1) sorting a bar-coded cDNA library using a nucleic acid array (optionally having many concave loci), the bar-code enabling sorting in physical space by hybridization to nucleic acid arrays complementary to the bar-code sequences;, (2) transfecting the sorted library into a readout cell line in situ, optionally by contacting the nucleic acid array with the cell line in the presence of a solution facilitating release of the bar-coded cDNA library; and, (3) conducting a biological readout assay., INDEPENDENT CLAIMS are also included for:, (1) a similar method, in which the library is sorted using a nucleic acid array having many concave loci, the sorted library is expressed using in vitro transcription and translation to produce a population of proteins, and the proteins are screened for an activity of interest; and, (2) similar methods not necessarily utilizing bar-coded cDNA libraries, comprising:, (a) conducting steps (b) and (c) on a presorted (optionally as in (a)) cDNA library; and, (b) expression cloning one or more genes of interest in a cDNA library by sorting the cDNA library (optionally using a nucleic acid array), transfecting the sorted library into a readout cell line and identifying a positive signal (optionally by immunocytochemistry) in a biological readout assay., USE - The assay is useful to identify and functionally characterize new genes, useful in gene therapy (e.g. for disorders associated with single genes e.g. hemophilia or multiple genes e.g. diabetes) and to identify specific diseases to optimize therapeutic intervention, e.g. in conditions involving several different diseases (e.g. dementia). Discovery of new genes using the assay is also useful to develop diagnostics for such diseases. The assay is especially useful to detect genes in specific (e.g. mitogenic, apoptosis, proliferation etc.; claimed) signaling pathways. The method of (1) is also useful to provide proteins which may be useful as drugs, e.g. with a receptor binding ligand binding or a growth factor activity (claimed)., ADVANTAGE - The assay allows functional screening of genes over days rather than weeks using previous functional screening techniques, and allows automated screening of many genes and their functions simultaneously, unlike prior art methods concentrating on individual genes.

L9: Entry 63 of 63 File: DWPI Nov 16, 1999

DERWENT-ACC-NO: 2000-086386
DERWENT-WEEK: 200015
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TITLE: New function-based gene discovery method, useful to identify genes for gene therapy

ABTX:
(2) transfecting the sorted library into a readout cell line in situ, optionally by contacting the nucleic acid array with the cell line in the presence of a solution facilitating release of the bar-coded cDNA library; and

ABTX:
(b) expression cloning one or more genes of interest in a cDNA library by sorting the cDNA library (optionally using a nucleic acid array), transfecting the sorted library into a readout cell line and identifying a positive signal (optionally by immunocytochemistry) in a biological readout assay.

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L15 ANSWER 38 OF 44 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:708928 CAPLUS
DOCUMENT NUMBER: 131:332950
TITLE: Function-based gene discovery using unique oligonucleotide-tagged bar-coded vectors for clone tracking and automation in cDNA library screening
INVENTOR(S): Cen, Hui; Sun, Shaojian
PATENT ASSIGNEE(S): Genova Pharmaceuticals Corporation, USA
SOURCE: PCT Int. Appl., 68 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9955886	A1	19991104	WO 1999-US8823	19990421

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

AU 9935727	A1	19991116	AU 1999-35727	19990421

PRIORITY APPLN. INFO.: US 1998-65775 19980424
WO 1999-US8823 19990421

AB The present invention relates generally to the field of genomics. More particularly, the present invention relates to methods for function-based gene discovery. Genes are identified as having or being assocd. with a specific function, as participating in a specific functional pathway, or as being a member of a specific functional group, by functional expression in one or more biol. readout assays. This invention is based, at least in part, on the recognition that the signal-to-noise ratio of a readout assay used to screen a cDNA library can be significantly enhanced by methods which localize multiple mol. copies of each unique clone into discrete regions or compartments prior to functional expression. In one embodiment, this invention provides methods for in situ transfection of a sorted library in a "bar-coded" vector to carry out expression of genes from libraries being screened in readout cells. The vector "bar code" is an oligonucleotide sequence within the vector which is unique to each individual clone of a library. The bar code enables sorting of the library in phys. space by hybridization to nucleic acid arrays which are complementary to library bar code sequences. The bar code unique to each clone together with the unique position of each complementary bar code in a nucleic acid array provides a method for direct retrieval of a gene having a function of interest in any given readout assay. Further, each unique bar code can serve as a specific primer for PCR and/or sequencing of a desired clone in a library. It is the ability to detect a biol. readout in a readout cell line which enables the user to identify genes having specific functions. It is able to directly screen mammalian cDNA libraries with an av. size of 106 clones through automation. Digestion of vectors is involved with restriction endonucleases. The methods set forth herein are suitable for application in a high throughput format for identification of genes and their functions simultaneously. Discovery of new genes and their functions permits development of diagnostics for early detection of diseases. This method permits discovery of discovery of disease-assocd. genes and is suitable for use with antisense libraries.

REFERENCE COUNT: 7
REFERENCE(S): (1) Affymetrix Inc; EP 0799897 A1 1997 CAPLUS
(2) Brenner; US 5604097 A 1997 CAPLUS
(3) Clark; US 4675285 A 1987 CAPLUS
(4) Fodor; US 5445934 A 1995 CAPLUS
(5) King; US 5654150 A 1997 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> s transfect?
L1 259010 TRANSFECT?

=> s immobiliz? or bound or bind? or adher?
L2 3294812 IMMOBILIZ? OR BOUND OR BIND? OR ADHER?

=> s dna or (nucleic(w)acid) or oligonucleotide? or polynucleotide? or vector? or plasmid?
L3 2793709 DNA OR (NUCLEIC(W) ACID) OR OLIGONUCLEOTIDE? OR POLYNUCLEOTIDE? OR VECTOR? OR PLASMID?

=> s array? or multiwell or (multiple(w)well)
L4 158024 ARRAY? OR MULTIWELL OR (MULTIPLE(W) WELL)

=> s l1 and l2 and l3 and l4
L5 328 L1 AND L2 AND L3 AND L4

=> s l2(l)l3
L6 424877 L2(L) L3

=> s l2(l)l4
L7 13254 L2(L) L4

=> s l1 and l6 and l7
L8 225 L1 AND L6 AND L7

=> dup rem
ENTER L# LIST OR (END):l8
PROCESSING COMPLETED FOR L8
L9 97 DUP REM L8 (128 DUPLICATES REMOVED)

=> s l9 and py<1999
1 FILES SEARCHED...
2 FILES SEARCHED...
L10 74 L9 AND PY<1999

=> d l10 ibib abs 1-74

L10 ANSWER 1 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1998:477851 BIOSIS
DOCUMENT NUMBER: PREV199800477851
TITLE: An array of binding sites for hepatocyte nuclear factor 4 of high and low affinities modulates the liver-specific enhancer for the human alpha1-microglobulin/bikunin precursor.
AUTHOR(S): Rouet, Philippe; Raguenez, Gilda; Ruminy, Philippe; Salier, Jean-Philippe (1)
CORPORATE SOURCE: (1) INSERM Unit-78, Fac. Med.-Pharm., 22 Blvd. Gambetta, 76000 Rouen France
SOURCE: Biochemical Journal, (Sept. 15, 1998) Vol. 334, No. 3, pp. 577-584.
ISSN: 0264-6021.
DOCUMENT TYPE: Article
LANGUAGE: English
AB alpha1-Microglobulin and bikunin are two plasma glycoproteins encoded by a gene for alpha1-microglobulin/bikunin precursor (AMBP). The strict liver-specific transcription of the AMBP gene is controlled by an elaborate and remote enhancer made of six clustered boxes numbered 1 to 6 (core enhancer) that are binding sites for the hepatocyte-enriched nuclear factors HNF-1, HNF4, HNF-3, HNF-1, HNF-3 and HNF-4 respectively. Three further boxes, 7 to 9, have now been found in the enhancer area in a position 5' of box 2, 5' of box 1 and 3' of box 6, respectively. Electrophoretic mobility-shift assays with nuclear extracts from the HepG2 hepatoma cell line demonstrated that boxes 7 and 8 are both functional HNF-4-binding sites of high and low affinity respectively, whereas no binding capacity of box 9 was detected by this method. Transfection of HepG2 and Chinese hamster ovary cells with chloramphenicol acetyltransferase constructs harbouring the core or extended AMBP enhancer with wild-type or mutated boxes and co-transfection with expression plasmids for a wild-type or defective HNF-4 identified box 7 as an essential element for the basal activity of this enhancer. The response of boxes 7 and 8 varies with the level of HNF-4 in cells. Box 9 exhibits a repressor activity that can be detected when box 8 is ablated. In vivo this corresponds to conditions of low box 8 occupancy when the intracellular level of HNF-4 is limited. These results reinforce the view that the AMBP enhancer is a quite elaborate and unusual example of a modular enhancer whose activity is fine-tuned by the level of cognate nuclear factors in the cell.

L10 ANSWER 2 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1998:361923 BIOSIS
DOCUMENT NUMBER: PREV199800361923
TITLE: Dominant negative regulation by c-Jun of transcription of the uncoupling protein-1 gene through a proximal cAMP-regulatory element: A mechanism for repressing basal and norepinephrine-induced expression of the gene before brown adipocyte differentiation.
AUTHOR(S): Yubero, Pilar; Barbera, M. Jose; Alvarez, Rosa; Vinas, Octavi; Mampel, Teresa; Iglesias, Roser; Villarroya, Francesc; Giralt, Marta (1)
CORPORATE SOURCE: (1) Dep. Bioquim. Biol. Mol., Univ. Barcelona, Avda. Diagonal 645, 08028 Barcelona Spain
SOURCE: Molecular Endocrinology, (July, 1998) Vol. 12, No. 7, pp. 1023-1037.
ISSN: 0888-8809.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The brown fat uncoupling protein-1 (ucp-1) gene is regulated by the sympathetic nervous system, and its transcription is stimulated by norepinephrine, mainly through cAMP-mediated pathways. Overexpression of the catalytic subunit of protein kinase A stimulated a chloramphenicol acetyltransferase expression vector driven by the 4.5-kb 5'-region of the rat ucp-1 gene. Mutant deletion analysis indicated the presence of the main cAMP-regulatory element (CRE) in the proximal region between -141 and -54. This region contains an element at -139/-122 able to confer enhancer and protein kinase A (PKA)-dependent activity to the basal thymidine kinase promoter. The potency of this element was much higher in differentiated than in nondifferentiated brown adipocytes. Gel shift analyses indicated that a complex array of proteins from brown fat nuclei bind to the -139/-122 element, among which CRE-binding protein (CREB) and Jun proteins were identified. In transfected brown adipocytes, c-Jun was a negative regulator of basal and PKA-induced transcription from the ucp-1 promoter acting through this proximal CRE region. A double-point mutation in the -139/-122 element abolished both PKA- and c-Jun-dependent regulation through this site, and overexpression of CREB blocked c-Jun repression. Thus, an opposite action of these two transcription factors on the -139/-122 CRE is proposed. c-Jun content in brown adipocytes differentiating in culture correlated negatively with both ucp-1 gene expression and the acquisition of the brown adipocyte morphology. These findings indicate that c-Jun provides a molecular mechanism to repress the basal and cAMP-mediated expression of the ucp-1 gene before the differentiation of the brown adipocyte.

L10 ANSWER 3 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1998:320723 BIOSIS
DOCUMENT NUMBER: PREV199800320723
TITLE: Sp1 and related factors fail to interact with the NF-kappaB-proximal G/C box in the LTR of a replication competent, brain-derived strain of HIV-1 (YU-2.
AUTHOR(S): Millhouse, Scott; Krebs, Fred C.; Yao, Jing; McAllister, John J.; Conner, Jean; Ross, Heather; Wigdahl, Brian (1)
CORPORATE SOURCE: (1) Pa. State Univ., Coll. Med., Dep. Microbiol. Immunol., Hershey, PA 17033 USA
SOURCE: Journal of Neurovirology, (June, 1998) Vol. 4, No. 3, pp. 312-323.
ISSN: 1355-0284.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The HIV-1 LTR promoter proximal G/C box array has been

demonstrated to
function by interacting with the Sp1 transcription factor family whose members can act as either activators or repressors of transcription. In this regard, we have examined the interaction of the HIV-1 Sp binding sites with nuclear factors that are present in cell types that support HIV-1 replication, including those of lymphocytic, monocytic, and astrocytic origin. As determined by electrophoretic mobility shift (EMS) competition analyses using oligonucleotides containing the sequences of each of the Sp1 sites of HIV-1 strain LAI, the NF-kappaB-proximal Sp site
(site III) displayed the highest binding activity compared to (Jurkat) and astrocytic (U-373 MG) nuclear extracts. Sp1 and two Sp3 isoforms were
detected as the primary cellular constituents of DNA-protein complexes formed with the NF-kappaB-proximal site. Only modest differences in Sp1:Sp3 binding ratios were observed when this site was reacted with either astrocytic or lymphocytic nuclear extract. However, when nuclear extracts derived from two-monocytic cell types that differ in the degree of differentiation were reacted with the HIV-1 LAI Sp site III, a large difference in Sp1 and Sp3 binding was observed. To determine if naturally occurring and replication-competent strains of HIV-1 contain base pair alterations within the Sp elements that affect the ability of the site to interact with Sp1 and related factors, a series of Sp site III variants were constructed and examined by EMS analyses. One of these sites, obtained from the published sequence of the YU-2 strain (a brain-derived macrophage tropic strain of HIV-1), displayed almost no Sp1
or Sp3 binding activity as a result of a single base pair alteration in Sp site III. This base-pair alteration, when placed in the context of an HIV-1 LAI LTR-luciferase construct, resulted in a 40-50% reduction in LTR
activity in transiently transfected Jurkat and U-373 MG cells. Overall, these results suggest that specific G/C box sequence alterations present in the brain-derived HIV-1 variant YU-2, or possibly other brain-derived variants, may exhibit altered replication properties as a result of the low affinity of the NF-kappaB-proximal G/C box for members of the Sp transcription factor family.

L10 ANSWER 4 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1998:296696 BIOSIS
DOCUMENT NUMBER: PREV199800296696
TITLE: Novel nuclear localization signal between the two DNA-binding zinc fingers in the human vitamin D receptor.
AUTHOR(S): Hsieh, Jui-Cheng (1); Shimizu, Yoshiko; Minoshima, Shinsei;
Shimizu, Nobuyoshi; Haussler, Carol A.; Jurutka, Peter W.; Haussler, Mark R.
CORPORATE SOURCE: (1) Dep. Biochem., Coll. Med., Univ. Arizona, Tucson, AZ
85724 USA
SOURCE: Journal of Cellular Biochemistry, (July 1, 1998) Vol. 70, No. 1, pp. 94-109.
ISSN: 0730-2312.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The human vitamin D receptor (hVDR) possesses a unique array of five basic amino acids positioned between the two DNA-binding zinc fingers that is similar to well-characterized nuclear localization sequences in other proteins. When residues within this region are mutated to nonbasic amino acids, or when this domain is deleted, the receptor is still well expressed, but it no longer associates with the vitamin D-responsive element in DNA, in vitro, and hVDR-mediated transcriptional activation is abolished in transfected cells. Concomitantly, the mutated hVDRs exhibit a significant shift in hVDR cellular distribution favoring cytoplasmic over nuclear retention as assessed by subcellular fractionation and immunoblotting. Independent immunocytochemical studies
employing a VDR-specific monoclonal antibody demonstrate that mutation or
deletion of this basic domain dramatically attenuates hVDR nuclear localization in transfected COS-7 cells. Although wild-type hVDR is partitioned predominantly to the nucleus in the absence of the 1,25-dihydroxyvitamin D3 (1,25(OH)2D3) hormone, treatment with ligand further enhances nuclear translocation, as it does to some degree in receptors with the basic region altered. The role of 1,25(OH)2D3 may be to
facilitate hVDR heterodimerization with retinoid X receptors, stimulating subsequent DNA binding and ultimately enhancing nuclear retention. Taken together, these data reveal that the region of hVDR between Arg-49 and Lys-55 contains a novel constitutive nuclear localization signal, RRSMKRK.

L10 ANSWER 5 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1998:271420 BIOSIS
DOCUMENT NUMBER: PREV199800271420
TITLE: Chromatin remodelling by the glucocorticoid receptor requires the BRG1 complex.
AUTHOR(S): Fryer, Christy J.; Archer, Trevor K. (1)
CORPORATE SOURCE: (1) Dep. Obstet. and Gynaecol., Univ. Western Ont., London
Regional Cancer Cent., 790 Commissioners Road East, London, ON N6A 4L6 Canada
SOURCE: Nature (London), (May 7, 1998) Vol. 393, No. 6680, pp. 88-91.
ISSN: 0028-0836.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The assembly of transcriptional regulatory DNA sequences into chromatin
plays a fundamental role in modulating gene expression. The promoter of the mouse mammary-tumour virus (MMTV) is packaged into a regular array
of nucleosomes when it becomes stably integrated into mammalian chromosomes, and has been used to investigate the relationship between chromatin architecture and transcriptional activation by the hormone-bound glucocorticoid and progesterone receptors. In mammalian cells that express both of these receptors, the progesterone receptor activates transcription from transiently transfected MMTV DNA, but not from organized chromatin templates. Moreover, the activated progesterone receptor inhibits the chromatin remodelling and consequent transcriptional stimulation that is mediated by the glucocorticoid receptor. Here we investigate the mechanism of this inhibition by characterizing the interaction of the glucocorticoid receptor with transcriptional co-activator and chromatin remodelling protein complexes. We show that when this receptor is prevented from interacting with the hBRG1/BAF chromatin remodelling complex, it can activate transcription from transiently transfected DNA but not from organized chromatin templates. Our results indicate that it may be possible to separate the transcriptional activation and chromatin remodelling activities of proteins that interact with hormone receptors.

L10 ANSWER 6 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1998:3562 BIOSIS
DOCUMENT NUMBER: PREV199800003562
TITLE: Overexpression of metallothionein decreases sensitivity of pulmonary endothelial cells to oxidant injury.
AUTHOR(S): Pitt, Bruce R. (1); Schwarz, Margaret; Woo, Elizabeth S.;
Yee, Emily; Wasserloos, Karla; Tran, Sothi; Weng, Weili; Mannix, Robert J.; Watkins, Simon A.; Tyurina, Yulia Y.; Tyurin, Vladimr A.; Kagan, Valerian E.; Lazo, John S.
CORPORATE SOURCE: (1) Dep. Pharmacol., Univ. Pittsburgh Sch. Med., Pittsburgh, PA 15261 USA
SOURCE: American Journal of Physiology, (Oct., 1997) Vol. 273, No. 4 PART 1, pp. L856-L865.
ISSN: 0002-9513.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Metallothionein (MT) is a low-molecular-weight cysteine-rich protein with
extensive metal binding capacity and potential nonenzymatic antioxidant activity. Despite the sensitivity of vascular endothelium to either heavy metal toxicity or oxidative stress, little is known regarding the role of MT in endothelial cells. Accordingly, we determined the sensitivity of cultured sheep pulmonary artery endothelial cells (SPAEC) that overexpressed MT to tert-butyl hydroperoxide (t-BOOH), hyperoxia, or 2,2'-azobis(2,4-dimethylvaleronitrile) (AMVN; peroxyl radical generator). Nontoxic doses of 10 muM Cd increased MT levels from 0.21 +- 0.03 to 2.07
+- 0.24 mug/mg and resulted in resistance to t-BOOH and hyperoxia as determined by reduction of Alamar blue or (3H)serotonin transport, respectively. SPAEC stably transfected with plasmids containing either mouse or human cDNA for MT were resistant to both t-BOOH and

hyperoxia. In addition, we examined transition metal-independent, noncytotoxic AMVN-induced lipid peroxidation after metabolic incorporation of the oxidant-sensitive fluorescent fatty acid cis-parinaric acid into phospholipids and high-performance liquid chromatography separation. SPAEC that overexpressed MT after gene transfer completely inhibited peroxyl oxidation of phosphatidylserine, phosphatidylcholine, and sphingomyelin (but not phosphatidylethanolamine) noted in wild-type SPAEC. These data show for the first time that MT can 1) protect pulmonary artery endothelium against a diverse array of prooxidant stimuli and 2) directly intercept peroxyl radicals in a metal-independent fashion, thereby preventing lipid peroxidation in intact cells.

L10 ANSWER 7 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1997:485763 BIOSIS
DOCUMENT NUMBER: PREV199799784966
TITLE: Multiple characteristics of a pentameric regulatory array endow the human alpha-subunit glycoprotein hormone promoter with trophoblast specificity and maximal activity.
AUTHOR(S): Budworth, Paul R.; Quinn, Patrick G.; Nilson, John H. (1)
CORPORATE SOURCE: (1) Dep. Pharmacol., Sch. Med., Case Western Reserve Univ., 2109 Adelbert Road, Cleveland, OH 44106-4965 USA
SOURCE: Molecular Endocrinology, (1997) Vol. 11, No. 11, pp. 1669-1680. ISSN: 0888-8809.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Trophoblast-specific expression of the human alpha-subunit glycoprotein hormone gene requires a tightly linked array of five different regulatory elements (trophoblast-specific element (TSE), alpha-activating element (alpha-ACT), a tandem cAMP response element (CRE), junctional regulatory element (JRE), and a CCAAT box). We examined their contextual contributions to trophoblast-specific expression by using transfection assays to evaluate activity of systematic block replacement mutations made within the 1500-bp 5'-flanking region of the human a-subunit gene. While all five elements were required for full activity, only the TSE and JRE displayed trophoblast specificity. Interestingly, the TSE-binding protein has limited tissue distribution whereas a JRE-binding protein appears trophoblast specific. Likewise, replacement studies with an AP-1 element that binds heterodimers of jun and fos indicated that this element was incapable of compensating for either the tandem CRE or JRE. This preference for both CRE- and JRE-binding proteins provides another avenue for configuring an alpha-subunit promoter with trophoblast specificity. Additional analysis with a cAMP response element binding protein (CREB)-Gal4 fusion protein further underscored the importance of CREB as well as suggested that transcriptional contributions come from both the DNA-binding domain and transactivation domain of this protein. We also examined the interactive nature of the pentameric array by placing a 15-bp random sequence between each element. Remarkably, only the insertion 3' of the CCAAT box diminished promoter activity. This suggested the absence of direct interactions between the transcriptional factors that bind each element in the array. It also suggested that the CCAAT box is position-dependent relative to the TATA box. This position dependence appeared cell-specific, as it was not manifest in a gonadotrope cell line (alpha-T3-1 cells). Thus, the CCAAT box also has tissue-specific characteristics that assist in targeting expression of the alpha-subunit gene to trophoblasts. Together, these data suggest that multiple characteristics of a complex pentameric array of regulatory elements endow the alpha-subunit promoter with trophoblast specificity and maximal activity.

L10 ANSWER 8 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1997:156732 BIOSIS
DOCUMENT NUMBER: PREV199799455935
TITLE: Specific binding sites for a pol III transcriptional repressor and Pol II transcription factor YY1 within the internucleosomal spacer region in primate Alu repetitive elements.
AUTHOR(S): Humphrey, Glen W.; Englander, Ella W.; Howard, Bruce H. (1)
CORPORATE SOURCE: (1) Lab. Molecular Growth Regulation, National Inst. Child Health Human Dev., National Inst. Health, Bethesda, MD 20892 USA
SOURCE: Gene Expression, (1996) Vol. 6, No. 3, pp. 151-168. ISSN: 1052-2166.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Alu interspersed repetitive elements Possess internal RNA polymerase III promoters that are transcribed in vitro and in transfected mouse cells but are nearly silent in human HeLa cells. Transcriptional repression of these elements is to some extent reversible, as pol III-dependent Alu expression can be induced with herpes simplex or adenovirus. To assess whether sequence-specific DNA binding proteins might contribute to Alu transcriptional silencing, we examined the internucleosomal spacer region surrounding the B box of the Alu pol III promoter in HeLa cell nuclei for evidence of proteins bound at specific sites in vivo. We identified a DNase I-hypersensitive site 5' to the B box and a DNase I-resistant region 3' to the B box in nuclei. An Alu-specific repressor binds to a 5-bp inverted repeat motif overlapping the 5' end of the TFIIIC binding site and may inhibit pol III transcription through competitive displacement. The level of Alu-specific pol III repressor activity is significantly reduced in adenovirus-infected HeLa cells, suggesting that the repressor may contribute to Alu transcriptional silencing in vivo. The 3' DNase I-resistant region coincided with a binding site for the pol II transcription factor YY1 in vitro. YY1 is one of the major proteins in HeLa cells having binding specificity for Alu elements. YY1 bound to tandem arrays of genomic Alu elements may play a role in chromatin organization and silencing.

L10 ANSWER 9 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1997:155988 BIOSIS
DOCUMENT NUMBER: PREV199799455191
TITLE: Cell-to-cell contact activates the long terminal repeat of human immunodeficiency virus 1 through its kappa-B motif.
AUTHOR(S): Faure, Eric (1); Lecine, Patrick; Lipcey, Carol; Champion, Serge; Imbert, Jean
CORPORATE SOURCE: (1) Inst. Chimie Biol., Case 31, Univ. Provence, Pl. V. Hugo, F-1331 Marseille Cedex 3 France
SOURCE: European Journal of Biochemistry, (1997) Vol. 244, No. 2, pp. 568-574. ISSN: 0014-2956.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Cell-to-cell contact between peripheral blood lymphocytes and transfected human colonic carcinoma cell line HT29 activates transcription of the long terminal repeats (LTR) of human immunodeficiency virus. HIV-1 LTR transcription is controlled by a complex array of virus-encoded and cellular proteins. Using various constructs expressing a lacZ reporter gene under the control of the intact or three deleted forms of HIV-1 LTR, we obtained evidence that the kappa-B regulatory elements located in the U3 region are involved in cell-to-cell activation of HIV-1 LTR. Cell-to-cell contact activates in vitro binding of the nuclear factor kappa-B (NF-kappa-B) p50/p65 heterodimer to an HIV-1 kappa-B oligonucleotide. Cell-to-cell contact activation of NF-kappa-B was only partially inhibited by 100 mu-M pyrrolidine dithiocarbamate and was not correlated with a significant decrease of cellular inhibitor kappa-B-alpha. NF-kappa-B nuclear activation was not detectable before 1 h after cell contact and was dependent on protein synthesis.

L10 ANSWER 10 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1997:152816 BIOSIS
DOCUMENT NUMBER: PREV199799452019
TITLE: Lack of constitutive activation or inactivation of the platelet-activating factor receptor by glutamate

substitution of alanine 230.
AUTHOR(S): Carlson, Steve A.; Chatterjee, Tapan K.; Fisher, Rory A. (1)
CORPORATE SOURCE: (1) Dep. Pharmacol., Univ. Iowa Coll. Med., Iowa City, IA 52242 USA
SOURCE: Receptors & Signal Transduction, (1996) Vol. 6, No. 2, pp. 111-120.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The platelet-activating factor (PAF) receptor (PAFR) is a G protein-coupled receptor (GPCR) that mediates a diverse array of biological responses to PAF. Recently, we provided evidence that the third intracellular domain (3i) of the rat PAFR (rPAFR) is a critical determinant in its coupling to phosphoinositide phospholipase C (PI PLC)-activating G proteins. In the present study, we assessed the potential role of a conserved alanine in the carboxyl-terminal region of 3i of the rPAFR in rPAFR signaling activity. Previous studies with the m5 muscarinic acetylcholine and human PAF receptors revealed that substitution of a carboxyl-terminal alanine was found to impair receptor-mediated PI PLC activation. Here we report the effects of the analogous nonconservative substitution of glutamate for alanine 230 of the rPAFR (rPAFR A230E) on receptor-mediated agonist binding and PI PLC activation following transient expression of the receptor cDNA. BHK cells transfected with a cDNA encoding the rPAFR A230E exhibited PAF-stimulated increases in inositol phosphate (IP) accumulation with no increase in basal levels of IPs. PAF-stimulated IP production in rPAFR transfectants was dependent on the amount of DNA transfected, although PAF provoked a larger increase in IPs in rPAFR transfectants than in rPAFRA230E transfectants in cells transfected with equal amounts of receptor cDNA. This latter finding apparently reflects differences in the transfection efficiency or expression of the wild-type and rPAFR A230E cDNAs because PAF produced indistinguishable effects on IP accumulation in rPAFR and rPAFR A230E transfectants expressing equivalent numbers of receptors. These results provide evidence for a nonconserved role of this conserved alanine in coupling of group I GPCRs to PI PLC-activating G proteins and also suggest that this residue has differential roles in regulating expression and signaling by rat and human PAFRs.

L10 ANSWER 11 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1997:150515 BIOSIS
DOCUMENT NUMBER: PREV199799449718
TITLE: Erythroid Kruppel-like factor (EKLF) contains a multifunctional transcriptional activation domain important for inter- and intramolecular interactions.
AUTHOR(S): Chen, Xiaoyong; Bieker, James J. (1)
CORPORATE SOURCE: (1) Brookdale Cent. Molecular Biol., Dep. Biochemistry, Mount Sinai Sch. Med., One Gustave L. Levy Place, New York, NY 10029 USA
SOURCE: EMBO (European Molecular Biology Organization) Journal, (1996) Vol. 15, No. 21, pp. 5888-5896.
ISSN: 0261-4189.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Erythroid Kruppel-like factor (EKLF) is a red cell-restricted transcriptional activator that plays a dominant role in establishing high levels of beta-globin gene expression during erythroid ontogeny. Although its DNA binding domain belongs to the well-studied class of Kruppel-like zinc fingers, its proline-rich activation region has not been thoroughly examined. We have analyzed this region by monitoring the functional effects of its mutagenesis upon EKLF activity in vivo and in vitro. First, using co-transfection assays, we find that the transactivation region contains discrete stimulatory and inhibitory subdomains. Second, in vitro binding assays indicate that the inhibitory domain exerts its effect in cis by interfering with DNA binding. Third, in vivo competition assays demonstrate that EKLF interacts with a positive-acting cellular factor, and that the domain responsible for this trans interaction lies within a 40 amino acid sequence that is coincident with the EKLF minimal transactivation domain. Finally, site-directed mutagenesis of this domain implies that conformation and/or phosphorylation status of its central core may be critical for such interactions. These results point towards posttranslational steric and/or allosteric control of EKLF function that may be important not just for its DNA binding ability, but also for its potential to interact with other proteins that fully establish the correct stereospecific array leading to efficient switching of beta-globin transcription during development.

L10 ANSWER 12 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1996:537786 BIOSIS
DOCUMENT NUMBER: PREV199699260142
TITLE: Essential role of NF-E2 in remodeling of chromatin structure and transcriptional activation of the epsilon-globin gene in vivo by 5' hypersensitive site 2 of the beta-globin locus control region.
AUTHOR(S): Gong, Qihui; McDowell, Jennifer C.; Dean, Ann (1)
CORPORATE SOURCE: (1) Build. 6, Room B1-08, 6 Center Drive, MSC 2715, Bethesda, MD 20892-2715 USA
SOURCE: Molecular and Cellular Biology, (1996) Vol. 16, No. 11, pp. 6055-6064.
ISSN: 0270-7306.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Much of our understanding of the process by which enhancers activate transcription has been gained from transient-transfection studies in which the DNA is not assembled with histones and other chromatin proteins as it is in the cell nucleus. To study the activation of a mammalian gene in a natural chromatin context in vivo, we constructed a minichromosome containing the human epsilon-globin gene and portions of the beta-globin locus control region (LCR). The minichromosomes replicate and are maintained at stable copy number in human erythroid cells. Expression of the minichromosomal epsilon-globin gene requires the presence of beta-globin LCR elements in cis, as is the case for the chromosomal gene. We determined the chromatin structure of the epsilon-globin gene in both the active and inactive states. The transcriptionally inactive locus is covered by an array of positioned nucleosomes extending over 1,400 bp. In minichromosomes with a mu-LCR or DNase I-hypersensitive site 2 (HS2) which actively transcribe the e-globin gene, the nucleosome at the promoter is altered or disrupted while positioning of nucleosomes in the rest of the locus is retained. All or virtually all minichromosomes are simultaneously hypersensitive to DNase I both at the promoter and at HS2. Transcriptional activation and promoter remodeling, as well as formation of the HS2 structure itself, depended on the presence of the NF-E2 binding motif in HS2. The nucleosome at the promoter which is altered upon activation is positioned over the transcriptional elements of the epsilon-globin gene, i.e., the TATA, CCAAT, and CACCC elements, and the GATA-1 site at -165. The simple availability of erythroid transcription factors that recognize these motifs is insufficient to allow expression. As in the chromosomal globin locus, regulation also occurs at the level of chromatin structure. These observations are consistent with the idea that one role of the beta-globin LCR is to maintain promoters free of nucleosomes. The restricted structural change observed upon transcriptional activation may indicate that the LCR need only make a specific contact with the proximal gene promoter to activate transcription.

L10 ANSWER 13 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1996:282770 BIOSIS
DOCUMENT NUMBER: PREV199699005126
TITLE: Transactivation of adenovirus E2-early promoter by E1A and E4 6/7 in the context of viral chromosome.
AUTHOR(S): Swaminathan, Sathyamangalam; Thimmapaya, Bayar (1)
CORPORATE SOURCE: (1) Robert H. Lurie Cancer Cent., Microbiology Immunology Dep., Northwestern Univ. Med. Sch., Chicago, IL 60611 USA
SOURCE: Journal of Molecular Biology, (1996) Vol. 258, No. 5, pp. 736-746.
ISSN: 0022-2836.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Transcription from adenovirus E2-early promoter is controlled by a unique

array of four cis-acting elements which include an atypical TBP site, two E2F sites present in an inverted orientation relative to each other, and an ATF site. In virus-infected cells, this promoter is transactivated by E1A and the E4 6/7 proteins. In addition, it is also stimulated by the DNA-binding protein (DBP) in transient transfection assays. Here we describe a genetic analysis of the E2 transcriptional regulation in the context of the viral chromosome. By using genetically engineered mutant adenoviruses we have determined the interrelationship between the different cis-acting elements of the E2-early promoter during basal transcription, the extent to which E1A and E4 6/7 contribute to the E2 promoter activation and the E2 promoter elements that respond to these transactivators. We show that at eight hours following infection, E1A can transactivate the promoter about 21-fold whereas E4 6/7 can induce the promoter by only fivefold. DBP does not induce the promoter in the chromosomal context. Our mutational analysis suggests that the unique architecture of the E2-early promoter necessitates the concerted interaction of all three host transcription factors with their cognate recognition elements to form a stable and functional transcription complex. E1A mediated transactivation is dependent on this stable basal transcription complex and transactivation may involve simultaneous interaction of E1A with each of the three transcription factors present in the multicomponent basal transcription complex. The E4 6/7 protein can transactivate the E2-early promoter in the absence of ATF presumably by promoting the DNA binding capacity of transcription factor E2F and thereby stabilizing the basal transcription complex. We discuss some of the possible protein-protein interactions that may take place at the level of the multicomponent transcriptional complex at the E2-early promoter during transcriptional activation and the discrepancies that arise when a promoter is analyzed in infection versus transfection assays.

L10 ANSWER 14 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1996:154785 BIOSIS
DOCUMENT NUMBER: PREV199698726920
TITLE: Comparing transcriptional activation and autostimulation by ZEBRA and ZEBRA/c-Fos chimeras.
AUTHOR(S): Kolman, John L.; Taylor, Naomi; Gradoville, Lyn; Countryman, Jill; Miller, George (1)
CORPORATE SOURCE: (1) Dep. Mol. Biophysics Biochem., Yale Univ. Sch. Medicine, New Haven, CT 06520 USA
SOURCE: Journal of Virology, (1996) Vol. 70, No. 3, pp. 1493-1504.
ISSN: 0022-538X.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The lytic cycle of Epstein-Barr virus (EBV) can be activated by transfection of the gene for ZEBRA, a viral basic-zipper (bZip) transcriptional activator. ZEBRA and cellular AP-1 bZip activators, such as c-Fos, have homologous DNA-binding domains, and their DNA-binding specificities overlap. Moreover, EBV latency can also be disrupted by phorbol esters, which act, in part, through AP-1 activators. It is not known whether ZEBRA and AP-1 factors play equivalent roles in the initial stages of reactivation. Here, the contribution of ZEBRA's basic DNA recognition domain to disruption of latency WAS analyzed by comparing ZEBRA with chimeric mutants in which the DNA recognition domain of ZEBRA was replaced with the analogous domain of c-Fos. Chimeric ZEBRA/c-Fos proteins overexpressed in Escherichia coli bound DNA with the specificity of c-Fos; they bound a heptamer AP-1 site and an octamer TPA response element (TRE). ZEBRA bound the AP-1 site and an array of ZEBRA response elements (ZREs). In assays with reporter genes, both ZEBRA and ZEBRA/c-Fos chimeric mutants activated transcription from Zp, a promoter of the ZEBRA gene (BZLF1) that contains the TRE and multiple ZRES. However, despite their capacity to activate reporters bearing Zp, neither ZEBRA nor the c-Fos chimeras activated transcription from Zp in the context of the intact latent viral genome. In contrast, ZEBRA but not ZEBRA/c-Fos chimeras activated Rp, a second viral promoter that controls ZEBRA expression. Hence, transcriptional autostimulation by transfected ZEBRA occurred preferentially at Rp. Both ZEBRA and the ZEBRA/c-Fos chimeras activated transcription from reporters with multimerized AP-1 sites. However, in the context of the virus, only ZEBRA activated the promoters of two early lytic cycle genes, BMRF1 and BMLF1, that contain an AP-1 site. Thus, overexpression of an activator that recognized AP-1 and TRE sites was not sufficient to activate EBV early lytic cycle genes.

L10 ANSWER 15 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1995:550747 BIOSIS
DOCUMENT NUMBER: PREV199698565047
TITLE: Co-expression in CHO cells of two muscle proteins involved in excitation-contraction coupling.
AUTHOR(S): Takekura, Hiroaki; Takeshima, Hiroshi; Nishimura, Seiichiro; Takahashi, Masami; Tanabe, Tsutomu; Flockerzi, Veit; Hofmann, Franz; Franzini-Armstrong, Clara (1)
CORPORATE SOURCE: (1) Dep. Cell. Dev. Biol., Univ. Pa., Sch. Med. Anat.-Chem. Build., Room B41, Philadelphia, PA 19104-6058 USA
SOURCE: Journal of Muscle Research and Cell Motility, (1995) Vol. 16, No. 5, pp. 465-480.
ISSN: 0142-4319.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Ryanodine receptors and dihydropyridine receptors are located opposite each other at the junctions between sarcoplasmic reticulum and either the surface membrane or the transverse tubules in skeletal muscle. Ryanodine receptors are the calcium release channels of the sarcoplasmic reticulum and their cytoplasmic domains form the feet, connecting sarcoplasmic reticulum to transverse tubules. Dihydropyridine receptors are L-type calcium channels that act as the voltage sensors of excitation-contraction coupling: they sense surface membrane and transverse tubule depolarization and induce opening of the sarcoplasmic reticulum release channels. In skeletal muscle, ryanodine receptors are arranged in extensive arrays and dihydropyridine receptors are grouped into tetrads, which in turn are associated with the four subunits of ryanodine receptors. The disposition allows for a direct interaction between the two sets of molecules. CHO cells were stably transformed with plasmids for skeletal muscle ryanodine receptors and either the skeletal dihydropyridine receptor, or a skeletal-cardiac dihydropyridine receptor chimera (CSk3) which can functionally substitute for the skeletal dihydropyridine receptor, in addition to plasmids for the alpha-2, beta and gamma subunits. RNA blot hybridization gave positive results for all components. Immunoblots, ryanodine binding, electron microscopy and exposure to caffeine show that the expressed ryanodine receptors forms functional tetrameric channels, which are correctly inserted into the endoplasmic reticulum membrane, and form extensive arrays with the same spacings as in skeletal muscle. Since formation of arrays does not require coexpression of dihydropyridine receptors, we conclude that self-aggregation is an independent property of ryanodine receptors. All dihydropyridine receptor-expressing clones show high affinity binding for dihydropyridine and immunolabeling with antibodies against dihydropyridine receptor. The presence of calcium currents with fast kinetics and immunolabeling for dihydropyridine receptors in the surface membrane of CSk3 clones indicate that CSk3-dihydropyridine receptors are appropriately targeted to the cell's plasmalemma. The expressed skeletal-type dihydropyridine receptors, however, remain mostly located within perinuclear membranes. In cells coexpressing functional dihydropyridine receptors and ryanodine receptors, no junctions between feet-bearing endoplasmic reticulum elements and surface membrane are formed, and dihydropyridine receptors do not assemble into tetrads. A separation between dihydropyridine receptors and ryanodine receptors is not unique to CHO cells, but is found also in cardiac muscle, in muscles of invertebrates and, under certain conditions, in skeletal muscle. We suggest that failure to form junctions in co-transfected CHO cell may be due to lack of an essential protein necessary either for the initial docking of the endoplasmic reticulum to the surface membrane or for maintaining the interaction between dihydropyridine receptors and ryanodine receptors. We also conclude that formation of tetrads requires a close interaction between dihydropyridine receptors and ryanodine receptors.

L10 ANSWER 16 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1995:453379 BIOSIS
DOCUMENT NUMBER: PREV199598467679
TITLE: Repression of the ovalbumin gene involves multiple negative

elements including a ubiquitous transcriptional silencer.
AUTHOR(S): Haecker, Sarah A. Ehlen; Muramastu, Tatsuo; Sensenbaugh,
Karl R.; Sanders, Michael M. (1)
CORPORATE SOURCE: (1) Dep. Biochem., Univ. Minnesota, 4-225 Millard Hall, 435
Delaware St., Minneapolis, MN 55455 USA
SOURCE: Molecular Endocrinology, (1995) Vol. 9, No. 9, pp. 1113-1126.
ISSN: 0888-8809.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Most eukaryotic genes are controlled by a complex array of cis-acting regulatory elements that modulate transcriptional activity. Two major regulatory elements reside in the chicken ovalbumin gene, a steroid-dependent regulatory element (SDRE, -892 to -780) and a negative regulatory element (NRE, -308 to -88). The SDRE is required for responsiveness to estrogen and glucocorticoid. The NRE appears to have the dual role of repressing transcription in the absence of steroids and of cooperating with the SDRE to activate transcription in the presence of steroids. The experiments described herein were designed to investigate the role of the NRE in repressing gene expression. Transfection of OvCAT fusion genes containing deletions in the NRE into primary oviduct cell cultures identified three elements (-308 to -256, -239 to -220, and -174 to -88) that repress transcription. Oligomers corresponding to portions of these elements also independently repress the viral thymidine kinase promoter. Interestingly, the element from -239 to -220 functions mechanistically as a silencer and shares sequence identity with silencers in other genes (TCTCTCCNA). Mobility shift studies indicated that all of the negative elements bind specific protein complexes from oviduct, none of which is appreciably affected by treatment with steroid hormones. However, oviduct-specific proteins bind to the regions from -280 to -252 and from -134 to -88, providing the first identification of potential tissue-specific elements in the ovalbumin gene. These results demonstrate that the region of DNA originally called the NRE is a multifunctional regulatory element that may be involved in several diverse regulatory activities.

L10 ANSWER 17 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1995:435834 BIOSIS
DOCUMENT NUMBER: PREV199598450134
TITLE: Regulation of insulin-like growth factor I transcription by cyclic adenosine 3',5'-monophosphate (cAMP) in fetal rat bone cells through an element within exon 1: Protein kinase A-dependent control without a consensus AMP response element.
AUTHOR(S): McCarthy, Thomas L. (1); Thomas, Michael J.; Centrella,
Michael; Rotwein, Peter
CORPORATE SOURCE: (1) Sect. Plastic Surgery, 333 Cedar St., P.O. Box 208041,
New Haven, CT 06520-8041 USA
SOURCE: Endocrinology, (1995) Vol. 136, No. 9, pp. 3901-3908.
ISSN: 0013-7227.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Insulin-like growth factor I (IGF-I) is a locally synthesized anabolic growth factor for bone. IGF-I synthesis by primary fetal rat osteoblasts (Ob) is stimulated by agents that increase the intracellular cAMP concentration, including prostaglandin E-2 (PGE-2). Previous studies with Ob cultures demonstrated that PGE-2 enhanced IGF-I transcription through selective use of IGF-I promoter 1, with little effect on IGF-I messenger RNA half-life. Transient transfection of Ob cultures with an array of promoter 1-luciferase reporter fusion constructs has now allowed localization of a potential cis-acting promoter element(s) responsible for cAMP-stimulated gene expression to the 5'-untranslated region (5'-UTR) of IGF-I exon 1, within a segment lacking a consensus cAMP response element.
Our evidence derives from three principal observations: 1) a transfection construct containing only 122 nucleotides (nt) of promoter 1 and 328 nt of the 5'-UTR retained full PGE-2-stimulated reporter expression; 2) maximal PGE-2-driven reporter expression required the presence of nt 196 to 328 of exon 1 when tested within the context of IGF-I promoter 1; 3) cotransfection of IGF-I promoter-luciferase-reporter constructs with a plasmid encoding the alpha-isoform of the catalytic subunit of murine cAMP-dependent protein kinase (PKA) produced results comparable to those seen with PGE-2 treatment, whereas cotransfection with a plasmid encoding a mutant regulatory subunit of PKA that cannot bind cAMP blocked PGE-2-induced reporter expression. Deoxyribonuclease I footprinting of the 5'-UTR of exon 1 demonstrated protected sequences at HS3A, HS3B, and HS3D, three of six DNA-protein binding sites previously characterized with rat liver nuclear extracts. Of these three regions, only the HS3D binding site is located within the functionally identified hormonally responsive segment of IGF-I exon 1. These results directly implicate PKA in the control of IGF-I gene transcription by PGE-2 and identify a segment of IGF-I exon 1 as being essential for this hormonal regulation.

L10 ANSWER 18 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1995:220307 BIOSIS
DOCUMENT NUMBER: PREV199598234607
TITLE: In vivo binding of proteins to stably integrated MMTV DNA in murine cell lines: Occupancy of NFI and OTF1 binding sites in the absence and presence of glucocorticoids.
AUTHOR(S): Hartig, Elisabeth; Cato, Andrew C. B. (1)
CORPORATE SOURCE: (1) Kernforschungszentrum Karlsruhe, Inst. Genetics, P.O.
Box 3640, D-76021 Karlsruhe Germany
SOURCE: Cellular & Molecular Biology Research, (1994) Vol. 40, No.
7-8, pp. 643-652.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Activation of expression at the mouse mammary tumor virus (MMTV) promoter is thought to be controlled by nucleosome positioning. On stably integrated MMTV DNA, the long terminal repeat (LTR) region is organized in a phased array of nucleosomes which allegedly occludes transcription factors such as NFI from binding. NFI only binds to the promoter region when the ordered nucleosome structure is apparently disrupted by activated steroid hormone receptors in hormone induced transcription. In certain cell fines, binding sites for the transcription factors NFI and OTF1 are however required for hormone-independent expression of MMTV. We have used stably transfected mouse NIH3T3 and GR cells that exhibit detectable MMTV expression in the absence of hormone for in vivo determination of proteins binding to the MMTV promoter. Here, we present in vivo dimethyl sulfate footprinting data that show that the NFI and OTF binding sites are permanently occupied in vivo in these cells. The contacting guanine residues identified in vivo were demonstrated in in vitro methylation interference assays to correspond to binding by NFI and OTF1. These results demonstrate a novel feature of transcription factor occupancy at the MMTV LTR promoter.

L10 ANSWER 19 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1995:203503 BIOSIS
DOCUMENT NUMBER: PREV199598217803
TITLE: Expression of alpha-1-Chimaerin (rac-1 GAP) Alters the Cytoskeletal and Adhesive Properties of Fibroblasts.
AUTHOR(S): Herrera, Roman (1); Shivers, Brenda D.
CORPORATE SOURCE: (1) Parke-Davis Pharm. Res. Div., 2800 Plymouth Rd., Ann
Arbor, MI 48105 USA
SOURCE: Journal of Cellular Biochemistry, (1994) Vol. 56, No. 4, pp. 582-591.
ISSN: 0730-2312.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The small GTP-binding protein rac-1, a member of the ras gene superfamily of GTPases, is thought to be a key component of a signal transduction pathway that mediates cell membrane ruffling and actin stress fiber formation induced by growth factors. rac-1 protein is regulated by the interplay of several activities: proteins that enhance GDP

dissociation (GDP Dissociation Stimulator, GDS), inhibit nucleotide exchange (GDP Dissociation Inhibitor, GDI), or accelerate GTP hydrolysis (GTPase Activating Protein, GAP). We have assessed the relative contribution of the rac-1/GAP interactions to the overall activity of rac-1 by expressing alpha-1-chimaerin, a rac-1-specific GAP, in fibroblasts. NIH 3T3 cells were transfected with alpha-1-chimaerin cDNA-containing expression vector and stable clones were established. Extracts prepared from alpha-1-chimaerin-expressing cells showed rac-1 GAP activity that was regulated by phosphatidylserine and phorbol ester. The cells expressing alpha-1-chimaerin showed a distinct phenotype. They had altered adhesive properties as measured by their ability to bind to a fibronectin-coated glass surface, suggesting that the expression of a rac-1 GAP alters the assembly of integrin receptors, actin and cytoskeletal proteins such as vinculin and talin. Direct demonstration of this phenomenon was achieved by studying the organization of actin stress fiber and formation of focal adhesions in the alpha-1-chimaerin expressing cells following stimulation by growth factors. Mock transfected cells, upon serum or lysophosphatidic acid stimulation, organize actin as a dense array of parallel fibers running the length of the cell. This process did not take place in the cells expressing rac-1 GAP. Similarly, the formation of focal adhesions as measured by the appearance of vinculin clusters was impaired in the alpha-1-chimaerin expressing cells. These results demonstrate that expression of a GAP for rac-1 in fibroblasts produces profound changes in the cytoskeletal organization and suggest that GAP activity negatively regulates rac-1 function.

L10 ANSWER 20 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1995:80600 BIOSIS
DOCUMENT NUMBER: PREV199598094900
TITLE: Interferon-stimulated response element and NF-kappa-B sites cooperate to regulate double-stranded RNA-induced transcription of the IP-10 gene.
AUTHOR(S): Wu, Chaoqun; Ohmori, Yoshihiro; Bandyopadhyay, Sudip; Sen, Ganes; Hamilton, Thomas (1)
CORPORATE SOURCE: (1) Cleveland Clinic Found., Dep. Immunol., 9500 Euclid Ave., Cleveland, OH 44195 USA
SOURCE: Journal of Interferon Research, (1994) Vol. 14, No. 6, pp. 357-363.
ISSN: 0197-8357.
DOCUMENT TYPE: Article
LANGUAGE: English
AB To understand the mechanisms involved in dsRNA-induced gene expression, we analyzed the poly(I/C)-induced transcription of the IFN-inducible chemokine gene IP-10 using the GRE cell line in which type I IFN genes have been deleted. Accumulation of IP-10 mRNA in GRE cells was more strongly stimulated by treatment with dsRNA than by IFN-alpha or IFN-gamma and was independent of protein synthesis. This same pattern of response was produced when GRE cells were transiently transfected with a plasmid containing 243 bases of sequence from the promoter of the murine IP-10 gene linked to the chloramphenicol acetyltransferase reporter gene. Deletion- and site-specific mutagenesis of the 243 base pair fragment indicated that an ISRE located between residues -204 and -228 was a primary target site for the action of dsRNA on this promoter. This was confirmed by results showing that two copies of this ISRE tandemly arrayed in front of the thymidine kinase promoter were able to mediate reporter gene transcription in dsRNA-stimulated cells. At least one of the two NF-kappa-B binding sites present in the 243 base pair IP-10 promoter is also necessary for response to dsRNA; mutation of both sites eliminates promoter activity. Thus the ISRE and one NF-kappa-B site cooperate to produce transcriptional response to dsRNA.

L10 ANSWER 21 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1994:531505 BIOSIS
DOCUMENT NUMBER: PREV199497544505
TITLE: Role of Anisomorphic DNA Conformations in the Negative Regulation of a Herpes Simplex Virus Type 1 Promoter.
AUTHOR(S): Sarisky, Robert T.; Weber, Peter C. (1)
CORPORATE SOURCE: (1) Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Exp. Ther. Dep., Infect. Dis. Sect., 2800 Plymouth Rd., Ann Arbor, MI 48105 USA
SOURCE: Virology, (1994) Vol. 204, No. 2, pp. 569-579.
ISSN: 0042-6822.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The a sequence is a bifunctional element in the herpes simplex virus type 1 (HSV-1) genome which possesses both the signals required for the cleavage and encapsidation of replicated viral DNA and the promoter-regulatory sequences for the gene encoding the viral neurovirulence factor ICP34.5. Since the ICP34.5 promoter locks features that are characteristic at most HSV-1 promoters, including a canonical TATA box, an initiator element, and upstream binding sites for host cell transcription factors, a mutational analysis was undertaken to identify the cis-acting elements which modiste transcription of this gone in transient transfection assays. A deletion derivative containing sequences just 83 nucleotides upstream of the second at two cap sites was found to exhibit full promoter activity. However, the presence of either of two for upstream regions, which coincided with the DR2 and DR6 tandem GC-rich repast arrays, acted to abrogate transcriptional activity both in this segment of the ICP34.5 promoter and in a heterologous promoter construct. The DR2 and DR6 repeat arrays each possessed an unwound S1 nuclease-sensitive DNA conformation (anisomorphic DNA) whose formation was shown to be critical for mediating this transcriptional repression effect. Moreover, results from in vivo titration experiments suggested the existence of a cellular protein(s) which can modiste transcriptional repression in the ICP34.5 promoter by specifically interacting with the single-stranded regions of these tandem repast arrays. Such DNA conformation-dependent transcriptional silencing appears to represent a novel mechanism of gone regulation in the HSV-1 life cycle.

L10 ANSWER 22 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1994:407376 BIOSIS
DOCUMENT NUMBER: PREV199497420376
TITLE: Specific steroid response from a nonspecific DNA element.
AUTHOR(S): Robins, D. M. (1); Scheller, A.; Adler, A. J.
CORPORATE SOURCE: (1) Dep. Human Genetics, Univ. Michigan Med. Sch., Med. Sci. II 4708, Ann Arbor, MI 48109-0619 USA
SOURCE: Journal of Steroid Biochemistry and Molecular Biology, (1994) Vol. 49, No. 4-6, pp. 251-255.
ISSN: 0960-0760.
DOCUMENT TYPE: Article
LANGUAGE: English
AB A fundamental dilemma of steroid hormone regulation is how specific transcription is attained in vivo when several receptors recognize the same DNA sequence in vitro. We have identified an enhancer of the mouse sex-limited protein (Slp) gene that is activated by androgens but not by glucocorticoids in transfection. Induction requires a consensus hormone response element (HRE) and multiple auxiliary elements within 120 base pairs. Androgen specificity relies on a dual function to augment androgen but prevent glucocorticoid action from a site that both receptors can bind. The nonreceptor factors are the dominant force in transcriptional specificity, although HRE sequence variations can affect the stringency and magnitude of hormonal response. The effect of HRE variations suggests that receptor position is altered relative to the other factors. Thus protein interactions that elicit specific gene regulation are established by the array of DNA elements in a complex enhancer and can be modulated by subtle sequence differences that may influence precise protein contacts.

L10 ANSWER 23 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1994:299867 BIOSIS
DOCUMENT NUMBER: PREV199497312867
TITLE: Differential steroid hormone induction of transcription from the mouse mammary tumor virus promoter.
AUTHOR(S): Archer, Trevor K.; Lee, Huay-Leng; Cordingley, Michael G.; Mymryk, Joe S.; Fragoso, Gilberto; Berard, Diana S.; Hager, Gordon L. (1)
CORPORATE SOURCE: (1) Hormone Action Oncogenesis Section, Lab.

Molecular
Virol., National Cancer Inst., Building 41, Room B405, Bethesda, MD 20892 USA
SOURCE: Molecular Endocrinology, (1994) Vol. 8, No. 5, pp. 568-576.
ISSN: 0888-8809.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The Mouse Mammary Tumor Virus (MMTV) contains sequences in its proximal promoter region to which both glucocorticoid and progesterone receptors can bind. In transient transfection experiments both hormones are able to stimulate transcription from reporter plasmids containing either native or consensus hormone response elements (glucocorticoid response element/progesterone response element). Previous experiments have demonstrated that the MMTV long terminal repeat is reproducibly assembled into a phased array of nucleosomes when stably introduced into cells. Stimulation by glucocorticoids of endogenous templates led to a rapid but transient increase in transcription initiation and mRNA accumulation that can be correlated with increased sensitivity to restriction enzymes. In contrast, experiments using progesterone or a truncated glucocorticoid receptor failed to elicit a similar increase in mRNA levels as dexamethasone from stable chromatin templates. In an attempt to understand this differential response, we have compared the responsiveness of the MMTV promoter to glucocorticoids and progesterone when it is organized in either stable chromatin or in transiently acquired plasmids. Our results demonstrate that the native chromatin structure prevents activation of this locus by progesterone, but permits stimulation by glucocorticoids.

L10 ANSWER 24 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1994:273608 BIOSIS
DOCUMENT NUMBER: PREV199497286608
TITLE: Molecular cloning and expression of the gene for a major leucine-rich protein from human hepatoblastoma cells (HepG2.
AUTHOR(S): Hou, Jinzhao; Wang, Fen; McKeehan, Walalce L.
CORPORATE SOURCE: W. Alton Jones Cell Sci. Cent. Inc., 10 Old Barn Road, Lake Placid, NY 12946 USA
SOURCE: In Vitro Cellular & Developmental Biology Animal, (1994) Vol. 30A, No. 2, pp. 111-114.
ISSN: 1071-2690.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The human hepatoblastoma cell line, HepG2, exhibits an array of stable properties in culture that have made it a popular cell culture model for studies on regulation of liver-specific gene expression and properties of hepatoma cells. In contrast to other hepatoma cell lines, HepG2 cells overexpress a characteristic detergent-extractable, wheat germ lectin-binding protein with apparent molecular mass of 130 kDa. Using an antibody to screen a phage expression library of HepG2 complementary DNA (cDNA), we identified and cloned a 4734 base pair cDNA which codes for a 130-kDa leucine-rich protein (lrp130) when expressed in transfected cells. The deduced sequence of lrp130 exhibits sequences weakly homologous to the consensus sequence for the ATP binding site in ATP-dependent kinases and the protein kinase C phosphorylation site of the epidermal growth factor receptor. Consistent with the higher levels of expression of lrp130 antigen, Northern hybridization analysis indicated that HepG2 cells express high levels of the major 4.8 kilobase lrp130 mRNA relative to other hepatoma cells. Although currently of unknown function, lrp130 may be of utility as a marker for liver cell lineages represented by the HepG2 cell line.

L10 ANSWER 25 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1993:522543 BIOSIS
DOCUMENT NUMBER: PREV199396135950
TITLE: The stringency and magnitude of androgen-specific gene activation are combinatorial functions of receptor and nonreceptor binding site sequences.
AUTHOR(S): Adler, Adam J.; Scheller, Arno; Robins, Diane M. (1)
CORPORATE SOURCE: (1) Dep. Human Genetics, University Michigan Medical Sch., Med. Sci. II 4708, Ann Arbor, MI 48109-0618 USA
SOURCE: Molecular and Cellular Biology, (1993) Vol. 13, No. 10, pp. 6326-6335.
ISSN: 0270-7306.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The mechanism by which specific hormonal regulation of gene expression is attained in vivo is a paradox in that several of the steroid receptors recognize the same DNA element in vitro. We have characterized a complex enhancer of the mouse sex-limited protein (Slp) gene that is activated exclusively by androgens but not by glucocorticoids in transfection. Potent androgen induction requires both the consensus hormone response element (HRE) and auxiliary elements residing within the 120-bp DNA fragment C'DELTA-9. Multiple nonreceptor factors are involved in androgen specificity, with respect to both the elevation of androgen receptor activity and the inactivity of glucocorticoid receptor (GR), since clustered base changes at any of several sites reduce or abolish androgen induction and do not increase glucocorticoid response. However, moving the HRE as little as 10 bases away from the rest of the enhancer allows GR to function, suggesting that GR is repressed by juxtaposition to particular factors within the androgen-specific complex. Surprisingly, some sequence variations of the HRE itself, within the context of C'DELTA-9, alter the stringency of specificity, as well as the magnitude, of hormonal response. These HRE sequence effects on expression correspond in a qualitative manner with receptor binding, i.e., GR shows a threefold difference in affinities for HREs amongst which androgen receptor does not discriminate. Altering the HRE orientation within the enhancer also affects hormonal stringency, increasing glucocorticoid but not androgen response. The effect of these subtle variations suggests that they alter receptor position with respect to other factors. Thus, protein-protein interactions that elicit specific gene regulation are established by the array of DNA elements in a complex enhancer and can be modulated by sequence variations within these elements that may influence selection of precise protein contacts.

L10 ANSWER 26 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1993:365701 BIOSIS
DOCUMENT NUMBER: PREV199396051376
TITLE: A steroid-inducible promoter for the controlled overexpression of cloned genes in eukaryotic cells.
AUTHOR(S): Mader, Sylvie; White, John H. (1)
CORPORATE SOURCE: (1) Dep. Physiology, McGill Univ., McIntyre Med. Sci. Build., 3655 Drummond Street, Montreal, PQ, Canada H3G 1Y6
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1993) Vol. 90, No. 12, pp. 5603-5607.
ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Previous studies have shown that members of the steroid receptor family of transcriptional regulators can function synergistically when bound to multiple arrays of specific DNA binding sites known as hormone response elements, usually located upstream of target genes. We have constructed a mammalian expression vector containing a synthetic promoter composed of five high-affinity glucocorticoid response elements (termed GRE5) placed upstream of the adenovirus 2 major late promoter "TATA" region. In transiently transfected HeLa cells in the presence of dexamethasone, the GRE5 promoter was at least 50-fold more efficient than the mouse mammary tumor virus long terminal repeat in expressing bacterial chloramphenicol acetyltransferase activity. When the GRE5 vector was introduced stably into the HeLa cell genome, chloramphenicol acetyltransferase activity was induced from 10- to gt 50-fold by dexamethasone in six of eight responsive clones. The levels of both basal

and induced expression varied from one clone to the next, probably due to an effect of chromosomal location on promoter activity. When propagated stably in HeLa cells in an Epstein-Barr virus episomal vector, the GRE5 promoter was gt 50-fold inducible and its activity was strictly dependent on the presence of dexamethasone. We also show that the GRE5 promoter stably propagated in HeLa cells is inducible by progesterone in the presence of a transiently transfected progesterone receptor expression vector. The GRE5 promoter should be widely applicable for the strictly controlled high-level expression of target genes in eukaryotic cells that contain either the glucocorticoid or progesterone receptors.

L10 ANSWER 27 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1993:365504 BIOSIS
DOCUMENT NUMBER: PREV199396051179
TITLE: A complex array of double-stranded and single-stranded DNA-binding proteins mediates induction of the ovalbumin gene by steroid hormones.
AUTHOR(S): Nordstrom, Lora A.; Dean, Diane M.; Sanders, Michel M.
CORPORATE SOURCE: Dep. Biochem., University Minnesota, Minneapolis, MN 55455 USA
SOURCE: Journal of Biological Chemistry, (1993) Vol. 268, No. 18, pp. 13193-13202.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The transcriptional induction of the chicken ovalbumin gene by steroid hormones is abolished by inhibitors of protein synthesis such as cycloheximide, suggesting that a labile protein mediates this process. A steroid-dependent regulatory element (SDRE) has been identified in the 5'-flanking region of the gene between -900 and -780 that is required for induction by steroids. Additional transfection experiments limit the 5'-border of the SDRE to the region between -892 and -864. To investigate whether any of the proteins binding to the SDRE are affected by estrogen or cycloheximide, protein binding was investigated using DNase I and exonuclease III footprinting and gel mobility shift assays. These experiments demonstrate that labile proteins bind to the sequences between -900 and -860 and between -810 and -820. Four oviduct nuclear proteins, including one of the labile proteins, binding to the SDRE prefer single-stranded DNA (ssDNA) in a sequence-specific manner. The binding activity of three of these ssDNA-binding proteins is increased in oviduct nuclear protein extracts from estrogen-treated chicks. These data suggest that induction of the ovalbumin gene is mediated by a complex collection of ssDNA- and double-stranded DNA-binding proteins whose activities are in turn regulated by their short half-lives or by estrogen.

L10 ANSWER 28 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1993:365475 BIOSIS
DOCUMENT NUMBER: PREV199396051150
TITLE: Characterization of protein-DNA interaction within the peroxisome proliferator-responsive element of the rat hydratase-dehydrogenase gene.
AUTHOR(S): Zhang, Baowei; Marcus, Sandra L.; Miyata, Kenji S.; Subramani, Suresh; Capone, John P.; Rachubinski, Richard A. (1)
CORPORATE SOURCE: (1) Dep. Biochem., McMaster Univ., Hamilton, ON Canada
SOURCE: Journal of Biological Chemistry, (1993) Vol. 268, No. 17, pp. 12939-12945.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB A peroxisome proliferator-responsive element is located in the 5'-flanking region of the gene encoding rat hydratase-dehydrogenase, the second enzyme of the peroxisomal 6-oxidation pathway. DNase I footprint analysis with nuclear extracts from proliferatorresponsive rat H4IIEC3 cells revealed two protected regions within the 196-base pair peroxisome proliferator-responsive element. Both regions contained multiple copies of a motif related to the consensus steroid hormone receptor binding half-site TGACCT, suggesting that peroxisome proliferator-dependent activation of this gene is mediated via peroxisome proliferator-activated receptors. Region II contains three TGACCT-like motifs in a direct repeat array. An oligonucleotide corresponding to this region was sufficient to confer responsiveness to the peroxisome proliferator ciprofibrate onto a heterologous promoter, as determined by transient transfection assays. Gel retardation assays demonstrated that nuclear factors bound to the hydratase-dehydrogenase oligonucleotide. Mutation of a single G residue within the second repeat motif abolished factor binding and consequently the ability of the element to respond to ciprofibrate, directly demonstrating that factor binding is necessary for peroxisome proliferator responsiveness. These results are discussed in the context of our current understanding of the mechanism of the coordinated transcriptional induction of the genes encoding peroxisomal beta-oxidation enzymes by peroxisome proliferators.

L10 ANSWER 29 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1993:165177 BIOSIS
DOCUMENT NUMBER: PREV199395086227
TITLE: The collagenous domains of macrophage scavenger receptors and complement component C1q mediate their similar, but not identical, binding specificities for polyanionic ligands.
AUTHOR(S): Acton, Susan; Resnick, David; Freeman, Mason; Ekkel, Yelena; Ashkenas, John; Krieger, Monty
CORPORATE SOURCE: Room E25-236, Biol. Dep., Massachusetts Inst. Technol., Cambridge, MA 02139 USA
SOURCE: Journal of Biological Chemistry, (1993) Vol. 268, No. 5, pp. 3530-3537.
ISSN: 0021-9258.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Macrophage scavenger receptors have been implicated in the development of atherosclerosis and other macrophage-associated functions, including host defense. The mechanism by which these receptors bind a wide array of polyanions, such as acetylated low density lipoprotein (AC-LDL), with high affinity has not yet been elucidated; however, it has been proposed that the positively charged extracellular collagenous domain of scavenger receptors plays a key role in ligand binding. To test this proposal, we generated truncation mutants of the bovine and murine scavenger receptors and studied their expression in transiently transfected COS cells. These mutants contain only 8 (bovine) or 5 (murine) of the 24 Gly-X-Y tripeptide repeats found in the collagenous domains of the full-length receptors. Immunochemical analyses established that the truncation of the bovine scavenger receptor did not interfere significantly with its synthesis, trimerization, post-translational processing, intracellular transport, surface expression, or stability. However, unlike their full-length counterparts, the truncated bovine and murine receptors were unable to bind Ac-LDL. Thus, the collagenous domain was necessary for normal ligand binding. In addition, cotransfection of the expression vector for the truncated bovine scavenger receptor with that for the full-length receptor resulted in dramatically reduced activity of the full-length construct (dominant negative effect). A ligand bead-binding assay was used to show that the isolated collagenous domain from a different protein, complement component C1q, could bind a wide variety of polyanions with a specificity which was similar, but not identical, to that of scavenger receptors. These results suggest that the collagenous domain of the scavenger receptor is both necessary and sufficient to determine the broad binding specificity that characterizes this unusual receptor. Scavenger receptors and C1q, along with the mannose-binding protein, conglutinin, and lung surfactant apoprotein A, help define a set of proteins which all contain short collagenous domains and which all appear to participate in host defense. Their short collagenous domains may contribute significantly to their host-defense functions.

L10 ANSWER 30 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1992:501553 BIOSIS
DOCUMENT NUMBER: BA94:120078
TITLE: TRANSCRIPTIONAL ACTIVATION OF THE TUMOR NECROSIS FACTOR ALPHA-INDUCIBLE ZINC FINGER PROTEIN A20 IS MEDIATED BY KAPPAB ELEMENTS.
AUTHOR(S): KRIKOS A; LAHERTY C D; DIXIT V M

CORPORATE SOURCE: DEP. PATHOLOGY, UNIVERSITY MICHIGAN SCHOOL MEDICINE, 1301
CATHERINE ST., P.O. BOX 0602, ANN ARBOR, MICH. 48109-0602.
SOURCE: J BIOL CHEM, (1992) 267 (25), 17971-17976.
CODEN: JBCHA3. ISSN: 0021-9258.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB A20 was first identified as a tumor necrosis factor (TNF) primary response
transcript encoding a 790-amino acid protein with a unique zinc finger motif. Recently, A230 was shown to protect cells from TNF-induced cytotoxicity in a variety of cell lines. Nuclear run-on studies previously established that TNF activates A20 at the transcriptional level. To further characterize the mechanism by which TNF activates the A20 gene, we
have cloned the A20 5'-flanking sequences and identified TNF-responsive elements within the promoter. The transcription initiation site was mapped by both primer extension and S1 nuclease protection experiments to a position 4.2 kilobases (kb) upstream of the initiator methionine; the first and second exon were separated by a 3.9-kb intron. Sequences upstream of the transcription start site were 76% GC-rich and contained six Sp1 binding sites and a TATA-like sequence at -29 but lacked a consensus CCAAT site. Transfection of Jurkat T-cells with an array of A20 promoter CAT constructs showed that two kB elements residing at -54 and -66 were required for induction by TNF. Supporting this notion, DNA electrophoretic mobility shift assays using nuclear extracts from unstimulated and TNF-stimulated Jurkat cells demonstrated kB-specific binding of a TNF-activated factor to an end-labeled probe containing the two A20 kB sequences. Finally, evidence obtained from cotransfection experiments showed that A20 negatively regulated its own expression.

L10 ANSWER 31 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1992:432459 BIOSIS
DOCUMENT NUMBER: BA94:84584
TITLE: STRUCTURE OF THE GENE AND ITS RETINOIC ACID-REGULATORY
REGION FOR MURINE J6 SERPIN AN F9 TERATOCARCINOMA CELL
RETINOIC ACID-INDUCIBLE PROTEIN.
AUTHOR(S): WANG S-Y
CORPORATE SOURCE: DEP. BIOLOGICAL SCIENCES, SUNY, ALBANY, N.Y. 12222.
SOURCE: J BIOL CHEM, (1992) 267 (22), 15362-15366.
CODEN: JBCHA3. ISSN: 0021-9258.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB We have recently reported a protein sequence deduced from the retinoic acid (RA)-inducible mRNA J6 as a novel serine protease inhibitor (serpin).
In this study we have reported that the J6 serpin gene is 7.7 kilobases in length and consists of five exons with an additional option. Comparison of the organization of the J6 gene and other serpin genes reveals that the structure of the J6 gene is different from the reported four serpin gene groups. Nonetheless, intron B of the J6 gene and members of the a-antitrypsin gene group are at the equivalent positions, suggesting that the J6 gene is more closely related to the members of a-antitrypsin gene group than other serpins. To identify the RA response region, we have further examined the nucleotide sequence of the 1-kilobase 5'-flanking region of the J6 gene. The DNA sequence from position - 1050 to -738 is essential for the gene activation by RA as revealed by the stable transfection experiments. Within this region, present are four GA-GATAG motifs which are the known binding sites for
GATA transcription factor family. Interestingly, there is a potential heat shock element with alternate arrays of blocks XGAAX and XTTCX spanning
from -88 to -59, indicating that the J6 gene perhaps is heat-inducible.

L10 ANSWER 32 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1990:308848 BIOSIS
DOCUMENT NUMBER: BA90:27815
TITLE: GLUCOCORTICOID RECEPTOR-DEPENDENT DISRUPTION OF A SPECIFIC
NUCLEOSOME ON THE MOUSE MAMMARY TUMOR VIRUS PROMOTER IS
PREVENTED BY SODIUM BUTYRATE.
AUTHOR(S): BRESNICK E H; JOHN S; BERARD D S; LEFEBVRE P; HAGER G L
CORPORATE SOURCE: HORMONE ACTION ONCOGENESIS SECT., LAB. EXPERIMENTAL
CARCINOGENESIS, NATL. CANCER INST., BETHESDA, MD. 20892.
SOURCE: PROC NATL ACAD SCI U S A, (1990) 87 (10), 3977-3981.
CODEN: PNASA6. ISSN: 0027-8424.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB Our laboratory has previously developed cell lines derived from mouse NIH
3T3 fibroblasts and C127 mammary tumor cells that stably express mouse mammary tumor virus (MMTV) long terminal repeat fusion genes in bovine
papillomavirus-based episomes. Glucocorticoid hormone strongly activates
transcription from episomes and induces the disruption of a single nucleosome in an array of phased nucleosomes on the MMTV promoter. Sodium butyrate inhibits the glucocorticoid hormone-dependent development
of a nuclease-hypertensive site that is due to the displacement of this nucleosome, and inhibits induction of RNA transcripts from episomes. Saturation binding studies show that butyrate treatment does not significantly affect the amount or the hormone-binding affinity of the glucocorticoid receptor. In a transient transfection assay, glucocorticoid hormone can activate transcription from a MMTV long terminal repeat-driven luciferase gene construct equivalently in untreated and butyrate-treated cells, indicating that the soluble factors necessary for transactivation of the MMTV promoter are unaffected by butyrate. The differential effect of butyrate on the induction of stable chromatin templates and transiently expressed plasmids suggests that butyrate prevents nucleosome displacement and repress transcription by inducing a modification of chromatin.

L10 ANSWER 33 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1990:178069 BIOSIS
DOCUMENT NUMBER: BA89:95239
TITLE: INTERSPECIFIC DNA-MEDIATED TRANSFER AND AMPLIFICATION OF A
GENE SPECIFYING A M-R 100000 HUMAN MELANOMA-ASSOCIATED CELL
SURFACE GLYCOPROTEIN.
AUTHOR(S): ROSENBERG C D; FERRONE S; HAMBY C V; MANCINO V; GRAF L H JR
CORPORATE SOURCE: UNIV. ILL. CHICAGO, CENT. RES. PERIDONTAL DISEASES ORAL
MOL. BIOL., 801 SOUTH PAULINA ST., CHICAGO, ILL. 60612.
SOURCE: CANCER RES, (1990) 50 (5), 1559-1565.
CODEN: CNREA8. ISSN: 0008-5472.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The gene that encodes the membrane-bound Mr 100,000 human melanoma-associated antigen (MAA) defined by mouse mAb 376.96, a leukocyte
and fibroblast interferon-modulated glycoprotein having preferential distribution on melanoma and carcinoma cells, has been transfected into the mouse melanoma cell line B78H1 as a step toward molecular cloning and
characterization of the MAA. Primary, secondary, and tertiary B78H1 transfectants expressing the Mr 100,000 MAA gene were generated by treatment with coprecipitated DNA from Mr 100,000 MAA+ human or transfectant mouse cell and they were detected by an indirect RBC rosetting assay. The Mr 100,000 MAA gene was also transferred into K-1735
mouse melanoma cells and into nonmalignant and malignant mouse fibroblast
lines. The species immunoprecipitated by mAB 376.96 from human melanoma
cells (Mr 100,000) and from mouse melanoma transfectant cells (Mr 97,000-100,000) were both converted to molecule(s) having an Mr of approximately 70,000 by enzymatic removal of asparagine-linked carbohydrate residues. Two independent secondary transformant clones of

B78H1 cells express Mr 100,000 MAA antigenicity at levels significantly higher than those observed when one or two copies of the gene are present.

Clone Mr 100,000 secondary-A spontaneously overexpresses Mr 100,000 MAA at least 5-fold and has ³10 times elevated levels of putatively Mr 100,000 MAA gene-associated human alu family repeat element (h-alu)-positive restriction fragments relative to "single" copy secondary transfectant cells. Clone Mr 100,000 secondary-B has increased copy number and expression of Mr 100,000 MAA as a consequence of a selective coamplification procedure which is targeted to a mouse wild type dihydrofolate reductase (dhfr) gene expression vector. This vector was cointroduced into B78H1 cells in addition to the DNA of Mr 100,000 MAA+ primary transfectant cells and the initially selected aminoglycoside phosphotransferase (neo) gene vector. Stepwise selections of a secondary Mr 100,000 MAA+ transfectant clone with increasing concentrations of the dihydrofolate reductase-inhibitory antimetabolite methotrexate led to progressive increases in copy numbers of the introduced dhfr gene and to parallel increases in h-alu sequences, in cellular levels of dihydrofolate reductase protein, and in cellular mAb 376.96 reactivity. Levels of these entities ultimately reached 50-fold, relative to levels expressed prior to amplification. The array of h-alu+ restriction fragments amplified in Mr 100,000 secondary-B cell DNA is very similar to that observed in Mr 100,000 secondary-A cell DNA. Co-amplified transfectant cells represent both an abundant source of the Mr 100,000 MAA molecule and an enriched source of the Mr 100,000 MAA genomic gene and of the corresponding mRNA; the latter are currently being used to facilitate isolation of recombinant clones related to this gene.

L10 ANSWER 34 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1987:101894 BIOSIS
DOCUMENT NUMBER: BA83:50872
TITLE: STRUCTURE AND FUNCTION OF HUMAN TISSUE-TYPE PLASMINOGEN ACTIVATOR.
AUTHOR(S): VAN ZONNEVELD A-J; VEERMAN H; MACDONALD M E; VAN MOURIK J A; PANNEKOEK H
CORPORATE SOURCE: DEP. MOL. BIOL., 1006 AK AMSTERDAM, NETH.
SOURCE: J CELL BIOCHEM, (1986) 32 (3), 169-178.
CODEN: JCEBD5. ISSN: 0730-2312.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB Full-length tissue-type plasminogen activator (t-PA) cDNA served to construct deletion mutants within the N-terminal "heavy" (H)-chain of the t-PA molecule. The H-chain cDNA consists of an array of structural domains homologous to domains present on other plasma proteins ("finger," "epidermal growth factor," "kringles"). These structural domains have been located on an exon or a set of exons. The endpoints of the deletions nearly coincide with exon-intron junctions of the chromosomal t-PA gene. Recombinant t-PA deletion mutant proteins were obtained after transient expression in mouse Ltk- cells, transfected with SV40-pBR322-derived t-PA cDNA plasmids. It is demonstrated that the serine protease moiety of t-PA and its substrate specificity for plasminogen is entirely contained within the C-terminal "light" (L)-chain of the protein. The presence of cDNA, encoding the t-PA signal peptide preceding the remaining portion of t-PA, suffices to achieve secretion of (mutant) t-PA into the medium. The stimulatory effect of fibrin on the plasminogen activator activity of t-PA was shown to be mediated by the kringle K2 domain and, to a lesser extent, by the finger domain. The other domains on the H-chain, kringle K1, and the epidermal growth-factor-like domain, do not contribute to this property of t-PA. These finding correlate well with the fibrin-binding properties of the rt-PA deletion-mutant proteins, indicating that stimulation of the activity is based on aligning of the substrate plasminogen and its enzyme t-PA on the fibrin matrix. The primary target for endothelial plasminogen activator inhibitor (PAI) is located within the L-chain of t-PA. Deleting specific segments of t-PA H-chain cDNA and subsequent transient expression in mouse Ltk- cells of t-PA deletion-mutant proteins did not affect the formation of a stable complex between mutant t-PA and PAI.

L10 ANSWER 35 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1986:377879 BIOSIS
DOCUMENT NUMBER: BA82:72855
TITLE: AUTONOMOUS FUNCTIONS OF STRUCTURAL DOMAINS ON HUMAN TISSUE-TYPE PLASMINOGEN ACTIVATOR.
AUTHOR(S): VAN ZONNEVELD A-J; VEERMAN H; PANNEKOEK H
CORPORATE SOURCE: CENT. LAB. NETHERLANDS RED CROSS BLOOD TRANSFUSION SERVICE, DEP. MOLECULAR BIOL., P.O. BOX 9406, 1006 AK AMSTERDAM, NETHERLANDS.
SOURCE: PROC NATL ACAD SCI U S A, (1986) 83 (13), 4670-4674.
CODEN: PNASA6. ISSN: 0027-8424.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB Transfected mouse Ltk- cells were employed for transient expression of recombinant human tissue-type plasminogen activator (t-PA; EC 3.4.21.31) or of recombinant-t-PA deletion proteins, encoded by SV40-pBR322-derived t-PA cDNA plasmids. The t-PA cDNA deletion mutants have two features in common, i.e., cDNA programming the signal peptide and the coding region for the light chain. Consequently, recombinant t-PA mutant proteins are efficiently secreted and display plasminogen activator activity. The gene encoding the amino-terminal heavy chain [an array of structural domains homologous to other plasma proteins (finger, epidermal growth factor, and kringle domains)] was mutated using restriction endonucleases to delete one or more structural domains. The stimulatory effect of fibrinogen fragments on the plasminogen activator activity of t-PA was demonstrated to be mediated by the kringle K2 domain and to a lesser extent by the finger/epidermal growth factor region but not by the kringle K1 domain. These data correlate well with the fibrin-binding properties of the recombinant t-PA deletion proteins, indicating that the stimulation of the activity by fibrinogen fragents is based on aligning the substrate plasminogen and t-PA onthe fibrin matrix. Our results support the evolutionary concept of exon shuffling, arranging structural domains that constitute autonomous functins of the protein.

L10 ANSWER 36 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1984:218172 BIOSIS
DOCUMENT NUMBER: BA77:51156
TITLE: TRANSLATION OF INSULIN RELATED POLY PEPTIDES FROM MESSENGER RNA WITH TANDEMLY REITERATED COPIES OF THE RIBOSOME BINDING SITE.
AUTHOR(S): KOZAK M
CORPORATE SOURCE: DEP. BIOL. SCI., UNIV. PITTSBURGH, PITTSBURGH, PA 15260.
SOURCE: CELL, (1983) 34 (3), 971-978.
CODEN: CELLB5. ISSN: 0092-8674.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB Plasmids were constructed containing reiterated copies of a 66 bp [base pair] fragment, loosely referred to as the ribosome binding site, that includes the AUG initiator codon of preproinsulin. The extreme test involved plasmid 255/17, which carried 4 tandem copies of the ribosome binding site, with all 4 AUG triplets in the same reading frame as the preproinsulin coding sequence downstream. Initiation at any potential start site would generate a polypeptide precipitable with anti-insulin antiserum, and its size would reveal the AUG(s) active in initiation. One insulin-related polypeptide was synthesized in cells transfected by p255/17; its size corresponded to the product initiated at the 1st ribosome binding site in the tandem array. Inasmuch as the 3 downstream AUG triplet, which are not used, occur in a sequence context identical with that around the 5'-proximal AUG triplets, which is used, the position of an AUG triplet relative to the 5' end of the mRNA appears

to be important in identifying it as a functional initiator codon.

L10 ANSWER 37 OF 74 BIOSIS COPYRIGHT 2001 BIOSIS

ACCESSION NUMBER: 1983:308627 BIOSIS
DOCUMENT NUMBER: BA76:66119
TITLE: LOSS OF POLYOMA VIRUS INFECTIVITY AS A RESULT OF A SINGLE
AMINO-ACID CHANGE IN A REGION OF POLYOMA VIRUS LARGE T
ANTIGEN WHICH HAS EXTENSIVE AMINO-ACID HOMOLOGY WITH SV-40
LARGE T ANTIGEN.
AUTHOR(S): HAYDAY A C; CHAUDRY F; FRIED M
CORPORATE SOURCE: IMPERIAL CANCER RES. FUND, LINCOLN'S INN FIELDS, LONDON
WC2A 3PX, UNITED KINGDOM.
SOURCE: J VIROL, (1983) 45 (2), 693-699.
CODEN: JOVIAM. ISSN: 0022-538X.
FILE SEGMENT: BA; OLD
LANGUAGE: English
AB The polyoma virus (Py) transformed cell line 7axB, selected by in vivo passage of an in vitro transformed [rat] cell, contains an integrated tandem array of 2.4 genomes and produces the large, middle and small Py T-antigen spp., with MW of 100,000, 55,000 and 22,000, respectively. The integrated viral and adjacent host DNA sequences have been molecularly cloned as 3 EcoRI fragments. One of these fragments (7B-M), derived from within the tandem viral sequences, is equivalent to an EcoRI viral linear molecule. Fragment 7B-M was found to be transformation competent but incapable of producing infectious virus after DNA transfection. By constructing chimerae betwen 7B-M and Py DNA and by direct DNA sequencing, the mutation responsible for the loss of infectivity has been located to a single base change (adenine to guanine) at nucleotide 2503. This results in a conversion of an aspartic acid to a glycine in the C-terminal region of the Py large T-antigen but does not appear to affect the binding of the Py large T-antigen to Py DNA at the putative DNA replication and autoregulation binding sites. The mutation is located within a 21-amino acid homology region shared by the SV40 large T-antigen. Evidently, the mutation in the 7axB large T-antigen may be involved in the active site of the protein for DNA replication.

L10 ANSWER 38 OF 74 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 97102611 EMBASE
DOCUMENT NUMBER: 1997102611
TITLE: Formation of de novo centromeres and construction of first-generation human artificial microchromosomes.
AUTHOR: Harrington J.J.; Van Bokkelen G.; Mays R.W.; Gustashaw K.; Willard H.F.
CORPORATE SOURCE: H.F. Willard, Center for Human Genetics, Case Western Reserve Univ. Med. Sch., Univ. Hospitals of Cleveland, Cleveland, OH 44106, United States. HFW@po.CWRU.edu
SOURCE: Nature Genetics, (1997) 15/4 (345-355).
Refs: 63
ISSN: 1061-4036 CODEN: NGENEC
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 022 Human Genetics
LANGUAGE: English
SUMMARY LANGUAGE: English
AB We have combined long synthetic arrays of alpha satellite DNA with telomeric DNA and genomic DNA to generate artificial chromosomes in human HT1080 cells. The resulting linear microchromosomes contain exogenous alpha satellite DNA, are mitotically and cytogenetically stable in the absence of selection for up to six months in culture, bind centromere proteins specific for active centromeres, and are estimated to be 6-10 megabases in size, approximately one-fifth to one-tenth the size of endogenous human chromosomes. We conclude that this strategy results in the formation of de novo centromere activity and that the microchromosomes so generated contain all of the sequence elements required for stable mitotic chromosome segregation and maintenance. This first-generation system for the construction of human artificial chromosomes should be suitable for dissecting the sequence requirements of human centromeres, as well as developing constructs useful for therapeutic applications.

L10 ANSWER 39 OF 74 EMBASE COPYRIGHT 2001 ELSEVIER SCI. B.V.

ACCESSION NUMBER: 92327623 EMBASE
DOCUMENT NUMBER: 1992327623
TITLE: NF-I/Sp1 switch elements regulate collagen a1(I) gene expression.
AUTHOR: Nehls M.C.; Grapilon M.L.; Brenner D.A.
CORPORATE SOURCE: Department of Medicine, University of California, 9500 Gilman Drive,San Diego, CA 92093-0623, United States
SOURCE: DNA and Cell Biology, (1992) 11/6 (443-452).
ISSN: 1044-5498 CODEN: DCEBE8
COUNTRY: United States
DOCUMENT TYPE: Journal; Article
FILE SEGMENT: 029 Clinical Biochemistry
LANGUAGE: English
SUMMARY LANGUAGE: English
AB The expression of type I collagen is regulated developmentally and tissue specifically. Two sets of binding sites for nuclear factor I (NF-I) and Sp1 transcription factors arrayed as an imperfect tandem repeat are critical for high activity of the murine a1(I) collagen gene in NIH-3T3 fibroblasts and are conserved in evolution. Gel retardation analysis combined with methylation interference studies show that NF-I and Sp1 bind to overlapping sites in a mutually exclusive manner. Cotransfection studies using Drosophila Schneider L2 cells, which lack both transcription factors, demonstrate that each factor alone trans-activates the gene, while cotransfection of both factors results in the inhibition of the strong Sp1 trans-activation. In contrast, the herpes simplex virus thymidine kinase promoter, which contains functionally independent NF-I and Sp1 binding sites, is maximally transactivated by the cotransfection of both factors. Because the two NF- I/Sp1 binding sites overlap, the ratio of the activities of the two factors rather than their absolute concentrations determine a1(I) gene expression, characterizing these promoter sequences as transcription factor switch elements.

L10 ANSWER 40 OF 74 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1998:756795 CAPLUS
DOCUMENT NUMBER: 130:77023
TITLE: Multiple cis-acting elements regulate angiotensinogen gene expression
AUTHOR(S): Zhao, Yan-Yan; Sun, Kai-Lai
CORPORATE SOURCE: Dep. Med. Genetics, China Med. Univ., Shenyang, 110001, Peop. Rep. China
SOURCE: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao (1998), 14(5), 492-497
CODEN: ZSHXF2; ISSN: 1007-7626
PUBLISHER: Zhongguo Shengwu Huaxue Yu Fenzi Shengwu Xuebao Bianweihui
DOCUMENT TYPE: Journal
LANGUAGE: Chinese
AB In order to understand the regulation of the human angiotensinogen gene expression, A 1220 bp fragment of the human angiotensinogen gene promoter that include 44 bp of the first exon was isolated. The fragment was directly cloned in the pGEM-T vector and subsequently subcloned through Sal I and Sph I restriction site in front of the chloramphenicol acetyl transferase (CAT) gene of pSVCAT-Basic vector. The 5'-sequential deletion mutants were obtained from the human angiotensinogen promoter attached to the CAT gene. An array of expression vectors were introduced into HepG2 and COS-7 cells by calcium phosphate pptn. transfection technique. The CAT activity was assayed using 14C-chloramphenicol as substrate. Results of transient transfection suggested two neg. regulatory fragments at -850~-580 and -420~-220 and two pos. regulatory fragments at-580~-420 and

-220~+1. Two synthetic oligonucleotides, homol. with IL-6 responsive element and estrogen responsive element in pos. regulatory fragments, were further analyzed by electrophoretic mobility shift assay and showed DNA-protein binding bands using nuclear ext. from COS-7 and HepG2 cells. Thus, expression of the human angiotensinogen gene is coordinately regulated by multiple cis-acting elements that interact with DNA binding proteins.

L10 ANSWER 41 OF 74 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:738274 CAPLUS
DOCUMENT NUMBER: 128:44283
TITLE: The role of nuclear matrix in tissue-specific gene expression
AUTHOR(S): Horton, Michael J.; Getzenberg, Robert H.
CORPORATE SOURCE: Department of Pathology, University of Pittsburgh, Pittsburgh, PA, USA
SOURCE: Adv. Mol. Cell Biol. (1997), 24(Cell Structure and Signaling), 185-206
CODEN: AMCBEZ
PUBLISHER: JAI Press
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review with 89 refs. Tissue-specific gene expression requires intricate sets of controls that are not yet fully understood. Mol. bases for specific gene controls have been sought among the chem. modification of DNA as well as alterations in the complement and arrangement of chromosomal proteins, but neither of these mechanisms correlate consistently with selective expression. Present models rely on the introduction of diversity into systems through the binding of an array of transcription factors including steroid hormone receptors, either as homodimeric or heterodimeric complexes, with a no. of DNA sequence motifs that act as regulatory sites in genes. However, the basic question of how cells of different tissues with an identical genetic makeup and that are responsive to the same nuclear factors, including hormone receptors, produce widely divergent secretory proteins remains unanswered. When viewed from a structural perspective, genes reside within the cellular nucleus in several levels of conformations from primary DNA sequence to higher order states in complex with chromatin proteins. At the highest conformational level, the nuclear matrix participates along with chromatin to organize genes into structural domains for control of their orderly expression and replication. The basic units of these structural domains are loops of chromosomal DNA attached to the nuclear matrix. Points of attachment consist of matrix-assocd. regions (MARs) of AT-rich DNA approx. 250 base pairs or greater in length and nuclear matrix proteins. Independent studies from several labs. have found MARs in 5', 3' and intronic sites of actively transcribed genes, whereas inactive genes are positioned away from anchorage points with the nuclear matrix. In transfection expts., some MARs have been shown to modulate transcription. Some studies have yielded a limited no. of tissue-specific matrix proteins that bind MARs, and in other reports the nuclear matrix has been found to bind consensus DNA regulatory elements and transcription factors. In contrast, some components of the nuclear matrix act in the repression of genes which may provide a mechanism for silencing of genetic domains. Thus, the nuclear matrix appears to delimit genetic regions for their expression or repression, according to tissue-specific requirements. Ongoing work from several labs. utilizing innovative model systems, esp. those that employ steroid hormone action, should further elucidate how the nuclear matrix might regulate tissue-specific gene expression.

L10 ANSWER 42 OF 74 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:476304 CAPLUS
DOCUMENT NUMBER: 127:105220
TITLE: Monitoring DNA binding molecules in living cells containing a steroid receptor response element array using a fluorescent chimeric protein of the steroid receptor
INVENTOR(S): Htun, Han; Hager, Gordon L.
PATENT ASSIGNEE(S): United States Dept. of Health and Human Services, USA; Htun, Han; Hager, Gordon L.
SOURCE: PCT Int. Appl., 99 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9720931	A1	19970612	WO 1996-US19516	19961206

W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG

CA 2239951	AA	19970612	CA 1996-2239951	19961206
AU 9712834	A1	19970627	AU 1997-12834	19961206

PRIORITY APPLN. INFO.: US 1995-8373 P 19951208
WO 1996-US19516 W 19961206

AB A method of screening for a compd. that binds to a selected nucleic acid is provided that comprises contacting a compd. fluorescently labeled by a fluorescent protein with a cell having a plurality of copies of the nucleic acid in an array such that the nucleic acid can be directly detected when bound by fluorescently labeled compd. The location of fluorescence within the cell is detected such that fluorescence aggregated at the site of the nucleic acid array indicates a compd. that binds to the selected nucleic acid. In particular compds. such a transcription factor can be screened. Reagents for such method are provided including a mammalian cell having a plurality of steroid receptor response elements in an array such that the response element can be directly detected when bound by fluorescently labeled steroid receptor and a chimeric protein comprising a fluorescent protein fused to a steroid receptor. Thus, a chimeric protein is constructed comprising a 27-kDa green fluorescent protein (GFP, from Aequorea victoria) and fused by a (Gly-Ala)5 peptide linker to the N-terminal second residue of rat glucocorticoid receptor (GR). Improved fluorescence is achieved by using a GFP variant contg. a serine-65 to threonine substitution, which increases the efficiency of formation of the GFP chromophore, and a GR variant contg. a cysteine-656 to glycine mutation has higher affinity for its ligand than endogenous receptor. A mammalian cell line named 3134 was derived by transfection of murine mammary carcinoma line C127 with a plasmid contg. 3 functional segments: (a) the bovine papilloma virus 69% transforming fragment serving as a replicon in mammalian cells; (b) mouse mammary tumor virus (MMTV) LTR is a steroid responsive promoter and contains the GR binding sites; and (c) the Ha-v-ras gene is a transforming oncogene and serves as a reporter for the MMTV promoter. The MMTV LTRs are organized in a head-to-tail tandem array of ~200 copies, and since each promoter sequence contains 4 GR binding sites, the complete array contains 100 GR binding sites. This cell is used to visualize directly the interaction between the fluorescent, chimeric GR and its binding site in chromatin in living cells. The system allows screening for DNA-binding ligands that (1) activate gene targeting by steroid receptor, (2) activate translocation of the steroid receptor to the nucleus, (3) are antagonists or agonists of the steroid receptor.

L10 ANSWER 43 OF 74 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:401375 CAPLUS
DOCUMENT NUMBER: 127:131852
TITLE: A tandem GC box motif is necessary for lipopolysaccharide-induced transcription of the type II TNF receptor gene
AUTHOR(S): Bethea, John, R.; Ohmori, Yoshihiro; Hamilton, Thomas A.

CORPORATE SOURCE: Dep. Immunology, Cleveland Clinic Foundation,
Cleveland, OH, 44195, USA
SOURCE: J. Immunol. (1997), 158(12), 5815-5823
CODEN: JOIMA3; ISSN: 0022-1767
PUBLISHER: American Association of Immunologists
DOCUMENT TYPE: Journal
LANGUAGE: English

AB LPs induces the expression of the gene encoding the type II TNF-a receptor in mononuclear phagocytes. To elucidate the nuclear signaling mechanisms involved in this response, a 772-bp fragment of the TNFRII gene promoter was analyzed by deletion and site-specific mutagenesis following transient transfection in the macrophage-like cell line RAW264.7. A region located between -100 and -75 relative to the transcription start site was found to be essential for LPs sensitivity. This contained a GC-rich region composed of two tandemly arrayed Sp-1 sites. While mutation of this region confirmed that it was essential for LPS sensitivity, the sequence was not able to confer LPS sensitivity upon a heterologous promoter. Internal deletion and site-specific mutagenesis of the 100-bp fragment identified regions immediately flanking an initiator region (Inr) site that were also necessary for sensitivity to LPS. Electrophoretic mobility shift assays demonstrated that the GC box bound Sp-1 and Sp-3, although the level of binding activity did not vary with LPS stimulation. An oligonucleotide probe contg. nucleotide positions -45 to -18 also bound several protein complexes, but these were not enhanced by LPS. These findings indicated that the tandem GC box is necessary, but not sufficient, for LPS-mediated transcription of the TNFRII gene. A second apparently novel motif, located within 15 nucleotides of the Inr, is also necessary.

L10 ANSWER 44 OF 74 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:21452 CAPLUS
DOCUMENT NUMBER: 126:153557
TITLE: T Antigen of human papovavirus JC stimulates transcription of the POU domain factor Tst-1/Oct6/SCIP
AUTHOR(S): Renner, Karin; Sock, Elisabeth; Gerber, Josef-Karl; Wegner, Michael
CORPORATE SOURCE: Zentrum Molekulare Neurobiologie, Univ. Hamburg,
Hamburg, D-20246, Germany
SOURCE: DNA Cell Biol. (1996), 15(12), 1057-1062
CODEN: DCEBE8; ISSN: 1044-5498
PUBLISHER: Liebert
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Human papovavirus JC exhibits a strong tropism for glial cells in vivo. To a large extent, this effect is due to the pronounced glia specificity of viral gene expression, which is mediated by the specific interaction of glial transcription factors such as Tst-1/Oct6/SCIP with viral promoter sequences. Here we show that, in return, expression of the glial transcription factor Tst-1/Oct6/SCIP can be strongly activated by T antigen, the early gene product of JC virus, in a dose-dependent manner. In transient transfection expts., stimulation by T antigen was entirely dependent on a 335-bp segment of the Tst-1/Oct6/SCI gene promoter that included the transcriptional start site. The same fragment was also bound by purified T antigen in immunopptn. assays due to the presence of three closely spaced and tandemly oriented GAGGC pentamers. However, when this array of pentamers was mutated so that binding of T antigen was strongly reduced, T-antigen-dependent transcriptional activation remained unaffected. Thus, similar to viral late gene expression, transcriptional stimulation of the Tst-1/Oct6/SCIP gene by T antigen was not dependent on binding to GAGGC pentamers present within the promoter. Nevertheless, our data provide strong support for a model in which JC virus influences gene expression of its host cell via its early gene product in a manner favorable for its own propagation.

L10 ANSWER 45 OF 74 CAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:445016 CAPLUS
DOCUMENT NUMBER: 122:257843
TITLE: In vivo binding of proteins to stably integrated MMTV DNA in murine cell lines: occupancy of NFI and OTF1 binding sites in the absence and presence of glucocorticoids
AUTHOR(S): Haertig, Elisabeth; Cato, Andrew C. B.
CORPORATE SOURCE: Inst. Genetics, Kernforschungszentrum Karlsruhe,
Karlsruhe, D-76021, Germany
SOURCE: Cell. Mol. Biol. Res. (1994), 40(7/8), 643-52
CODEN: CMBREW; ISSN: 0968-8773
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Activation of expression at the mouse mammary tumor virus (MMTV) promoter is thought to be controlled by nucleosome positioning. On stably integrated MMTV DNA, the long terminal repeat (LTR) region is organized in a phased array of nucleosomes which allegedly occludes transcription factors such as NFI from binding. NFI only binds to the promoter region when the ordered nucleosome structure is apparently disrupted by activated steroid hormone receptors in hormone induced transcription. In certain cell lines, binding sites for the transcription factors NFI and OTFI and OTF1 are however required for hormone-independent expression of MMTV. The authors have used stably transfected mouse NIH3T3 and GR cells that exhibit detectable MMTV expression in the absence of hormone for in vivo detn. of proteins binding to the MMTV promoter. Here, the authors present in vivo di-Me sulfate footprinting data that show that the NFI and OTF binding sites are permanently occupied in vivo in these cells. The contacting guanine residues identified in vivo were demonstrated in in vitro methylation interference assays to correspond to binding by NFI and OTF1. These results demonstrate a novel feature of transcription factor occupancy at the MMTV LTR promoter.

L10 ANSWER 46 OF 74 MEDLINE

ACCESSION NUMBER: 1999115387 MEDLINE
DOCUMENT NUMBER: 99115387 PubMed ID: 9914372
TITLE: Assay of centromere function using a human artificial chromosome.
AUTHOR: Masumoto H; Ikeno M; Nakano M; Okazaki T; Grimes B; Cooke H; Suzuki N
CORPORATE SOURCE: Division of Biological Science, Graduate School of Science,
Nagoya University, Chikusa-ku, Nagoya 464-8602, Japan..
g44478a@nucc.cc.nagoya-u.ac.jp
SOURCE: CHROMOSOMA, (1998 Dec) 107 (6-7) 406-16.
Journal code: D7A; 2985138R. ISSN: 0009-5915.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990324
Last Updated on STN: 19990324
Entered Medline: 19990311

AB In order to define a functional human centromere sequence, an artificial chromosome was constructed as a reproducible DNA molecule. Mammalian telomere repeats and a selectable marker were introduced into yeast artificial chromosomes (YACs) containing alphoid DNA from the centromere region of human chromosome 21 in a recombination-deficient yeast host. When these modified YACs were introduced into cultured human cells, a YAC with the alphoid DNA from the alpha21-I locus, containing CENP-B boxes at a high frequency and a regular repeat array, efficiently formed minichromosomes that were maintained stably in the absence of selection and bound CENP-A, CENP-B, CENP-C and CENP-E. The minichromosomes, 1-5 Mb in size and composed of multimers of the introduced YAC DNA, aligned at metaphase plates and segregated to opposite poles correctly in anaphase. Extensive cytological analyses strongly suggested that the minichromosomes had not acquired host sequences and were formed in all cases by a de novo mechanism. In contrast, minichromosomes were never produced with a

modified YAC containing alphoid DNA from the alpha21-II locus, which contains no CENP-B boxes and has a less regular sequence arrangement. We conclude that alpha21-I alphoid DNA can induce de novo assembly of active centromere/kinetochore structures on minichromosomes.

L10 ANSWER 47 OF 74 MEDLINE

ACCESSION NUMBER: 1998342041 MEDLINE
DOCUMENT NUMBER: 98342041 PubMed ID: 9678901
TITLE: Molecular heterogeneity of regulatory elements of the mouse GATA-1 gene.
AUTHOR: Ronchi A; Ciro M; Cairns L; Basilico L; Corbella P; Ricciardi-Castagnoli P; Cross M; Ghysdael J; Ottolenghi S
CORPORATE SOURCE: Dipartimento di Genetica e di Biologia dei Microrganismi, Universita di Milano, Italy.
SOURCE: GENES AND FUNCTION, (1997 Nov) 1 (4) 245-58. Journal code: C41; 9706385. ISSN: 1360-7413.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199808
ENTRY DATE: Entered STN: 19980828
Last Updated on STN: 19980828
Entered Medline: 19980818

AB The GATA-1 gene encodes a transcription factor expressed in early multipotent haemopoietic progenitors, in more mature cells of the erythroid, megakaryocytic and other lineages, but not in late myeloid precursors; its function is essential for the normal development of the erythroid and megakaryocytic system. To define regulatory elements of the mouse GATA-1 gene, we mapped DNaseI-hypersensitive sites in nuclei of erythroid and haemopoietic progenitor cells. Five sites were detected. The two upstream sites, site 1 and site 2, represent a new and a previously defined erythroid enhancer respectively. The site 1 enhancer activity depends both on a GATA-binding site (also footprinted in vivo) and on several sites capable of binding relatively ubiquitous factors. A DNA fragment encompassing site 1, placed upstream of a GATA-1 minimal promoter, is able to drive expression of a simian virus 40 (SV40) T-antigen in the yolk sac, but not bone-marrow cells, obtained from mice transgenic for this construct, allowing in vitro establishment of immortalized yolk-sac cells. A similar construct including site 2, instead of site 1, and previously shown to be able to immortalize adult marrow cells is not significantly active in yolk-sac cells. Sites 4 and 5, located in the first large intron, have no enhancer activity; they include a long array of potential Ets-binding sites. MnlI restriction sites, overlapping some of the Ets sites, are highly accessible, in intact nuclei, to MnlI. Although these sites are present in all GATA-1-expressing cells studied, they are the only strong sites detectable in FDCP-mix multipotent progenitor cells, most of which do not yet express GATA-1. The data indicate that appropriate GATA-1 regulation may require the co-operation of different regulatory elements acting at different stages of development and cell differentiation.

L10 ANSWER 48 OF 74 MEDLINE

ACCESSION NUMBER: 1998151509 MEDLINE
DOCUMENT NUMBER: 98151509 PubMed ID: 9482882
TITLE: Presence and phosphorylation of transcription factors in developing dendrites.
AUTHOR: Crino P; Khodakhah K; Becker K; Ginsberg S; Hemby S; Eberwine J
CORPORATE SOURCE: Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA.
CONTRACT NUMBER: AG9900 (NIA)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1998 Mar 3) 95 (5) 2313-8. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199804
ENTRY DATE: Entered STN: 19980416
Last Updated on STN: 19980416
Entered Medline: 19980409

AB In screening amplified poly(A) mRNA from hippocampal dendrites and growth cones in culture to determine candidates for local translation, we found that select transcription factor mRNAs were present. We hypothesized that synthesis of transcription factor proteins within dendrites would provide a direct signaling pathway between the distal dendrite and the nucleus resulting in modulation of gene expression important for neuronal differentiation. To evaluate this possibility, radiolabeled amplified antisense RNA was used to probe slot blots of transcription factor cDNAs as well as arrayed blots of zinc finger transcription factors. The mRNAs encoding the cAMP response element binding protein (CREB), zif 268, and one putative transcription factor were detected. We expanded upon these results showing that CREB protein is present in dendrites, that translation of CREB mRNA in isolated dendrites is feasible and that CREB protein found in dendrites can interact with the cis-acting cyclic AMP reponse element DNA sequence by using an in situ Southwestern assay. Further, CREB protein in dendrites is not transported to this site from the cell body because fluorescently tagged CREB microperfused into the soma did not diffuse into the dendrites. In addition, CREB protein microperfused into dendrites was rapidly transported to the nucleus, its likely site of bioactivity. Lastly, by using the isolated dendrite system we show that phosphorylation of Ser-133 on CREB protein can occur in isolated dendrites independent of the nucleus. These data provide a regulatory pathway in which transcription factors synthesized and posttranslationally modified in dendrites directly alter gene expression bypassing the integration of signal transduction pathways that converge on the nucleus.

L10 ANSWER 49 OF 74 MEDLINE

ACCESSION NUMBER: 1998112386 MEDLINE
DOCUMENT NUMBER: 98112386 PubMed ID: 9452007
TITLE: Structural characterization of myelin-associated glycoprotein gene core promoter.
AUTHOR: Laszkiewicz I; Grubinska B; Wiggins R C; Konat G W
CORPORATE SOURCE: Department of Anatomy, West Virginia University School of Medicine, Morgantown 26505-9128, USA.
SOURCE: JOURNAL OF NEUROSCIENCE RESEARCH, (1997 Dec 15) 50 (6) 928-36. Journal code: KAC; 7600111. ISSN: 0360-4012.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199803
ENTRY DATE: Entered STN: 19980312
Last Updated on STN: 19980312
Entered Medline: 19980304

AB Myelin-associated glycoprotein (MAG) is emerging as an important molecule involved in the plasticity and regeneration of the central nervous system. In this study, the structure of MAG gene promoter was characterized in cultured rat oligodendrocyte lineage cells. Heterogeneous transcription initiation with five major and eight minor start sites scattered within 72 bp was shown by primer extension analysis. This TATA-less core promoter contains no prominent initiator (Inr) elements associated with the transcription initiation sites, and hence, appears to utilize novel positioning mechanisms. Genomic footprinting analysis revealed several putative protein-binding regions overlapping the initiation sites and containing a multitude of CG-rich sequences. However, no conspicuous alterations in the protein-binding pattern were evident between O2A progenitors in which the gene is inactive, and mature oligodendrocytes with fully upregulated gene. The core promoter DNA features a differentiation-dependent demethylation as shown by genomic sequencing analysis. Three of eight cytosines are totally demethylated in oligodendrocyte chromosomes, indicating that these unmodified bases may be critical for full activation of the promoter. The core promoter is located

within an internucleosomal linker, and the upstream regulatory region appears to be organized into an array of nucleosomes with hypersensitive linkers.

L10 ANSWER 50 OF 74 MEDLINE

ACCESSION NUMBER: 97467741 MEDLINE
DOCUMENT NUMBER: 97467741 PubMed ID: 9326950
TITLE: Human telomeres contain two distinct Myb-related proteins, TRF1 and TRF2.
AUTHOR: Broccoli D; Smogorzewska A; Chong L; de Lange T
CORPORATE SOURCE: Rockefeller University, New York, New York 10021, USA.
CONTRACT NUMBER: 5 T32 GM07739 (NIGMS)
SOURCE: NATURE GENETICS, (1997 Oct) 17 (2) 231-5.
Journal code: BRO; 9216904. ISSN: 1061-4036.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF002999; GENBANK-AF003000; GENBANK-AF003001
ENTRY MONTH: 199711
ENTRY DATE: Entered STN: 19971224
Last Updated on STN: 20000303
Entered Medline: 19971114
AB Human telomeres are composed of long arrays of TTAGGG repeats that form a nucleoprotein complex required for the protection and replication of chromosome ends. One component of human telomeres is the TTAGGG repeat binding factor 1 (TRF1), a ubiquitously expressed protein, related to the protooncogene Myb, that is present at telomeres throughout the cell cycle. Recent evidence has implicated TRF1 in the control of telomere length. TRF1 is proposed to be an inhibitor of telomerase, acting in cis to limit the elongation of individual chromosome ends. Here we report the cloning of TRF2, a distant homologue of TRF1 that carries a very similar Myb-related DNA-binding motif. Like TRF1, TRF2 was ubiquitously expressed, bound specifically to duplex TTAGGG repeats in vitro and localized to all human telomeres in metaphase chromosomes. TRF2 was shown to have an architecture similar to that of TRF1 in that it carries a C-terminal Myb motif and a large TRF1-related dimerization domain near its N terminus. However, the dimerization domains of TRF1 and TRF2 did not interact, suggesting that these proteins exist predominantly as homodimers. While having similar telomere binding activity and domain organization, TRF2 differed from TRF1 in that its N terminus was basic rather than acidic, and TRF2 was much more conserved than TRF1. The results indicate that the TTAGGG repeat arrays at the ends of human and mouse chromosomes bind to two related proteins. Because TRF1 and TRF2 showed significant differences, we suggest that these factors have distinct functions at telomeres.

L10 ANSWER 51 OF 74 MEDLINE

ACCESSION NUMBER: 97465817 MEDLINE
DOCUMENT NUMBER: 97465817 PubMed ID: 9359410
TITLE: Activator-protein-1 binding potentiates the hypoxia-inducible factor-1-mediated hypoxia-induced transcriptional activation of vascular-endothelial growth factor expression in C6 glioma cells.
AUTHOR: Damert A; Ikeda E; Risau W
CORPORATE SOURCE: Max-Planck-Institut fur physiologische und klinische Forschung, W.G. Kerckhoff-Institut, Abteilung Molekulare Zellbiologie, Parkstrasse 1, 61231 Bad Nauheim, Germany.
SOURCE: BIOCHEMICAL JOURNAL, (1997 Oct 15) 327 (Pt 2) 419-23.
Journal code: 9YO; 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199712
ENTRY DATE: Entered STN: 19980109
Last Updated on STN: 19980109
Entered Medline: 19971208
AB The endothelial cell-specific mitogen vascular-endothelial growth factor (VEGF) plays a key role in both physiological and pathological angiogenesis. The up-regulation of VEGF expression in response to reduced oxygen tension occurs through transcriptional and post-transcriptional mechanisms. To investigate the molecular mechanisms of transcriptional activation by hypoxia (1% oxygen), fine mapping of a hypoxia-responsive region of the human VEGF promoter was carried out using luciferase reporter-gene constructs in C6 glioma cells. Here, we report that the binding site of hypoxia-inducible factor 1 (HIF1) is crucial for the hypoxic induction of VEGF gene expression. However, an enhancer subfragment containing the HIF1 binding site was not sufficient to confer full hypoxia responsiveness. Addition of upstream sequences restored the full sensitivity to hypoxia induction. This potentiating effect is due to activator protein 1 binding. The 'potentiating' sequences are unable to confer hypoxia responsiveness on their own. Our results strongly suggest that in C6 glioma cells a complex array of trans-acting factors facilitates full transcriptional induction of VEGF gene expression by hypoxia.

L10 ANSWER 52 OF 74 MEDLINE

ACCESSION NUMBER: 97382219 MEDLINE
DOCUMENT NUMBER: 97382219 PubMed ID: 9233988
TITLE: Hamster chromosomes containing amplified human alpha-satellite DNA show delayed sister chromatid separation in the absence of de novo kinetochore formation.
AUTHOR: Warburton P E; Cooke H J
CORPORATE SOURCE: MRC Human Genetics Unit, Western General Hospital, Edinburgh, EH4 2XU, UK.. peterwar@srv0.bio.ed.ac.uk
SOURCE: CHROMOSOMA, (1997 Aug) 106 (3) 149-59.
Journal code: D7A; 2985138R. ISSN: 0009-5915.
PUB. COUNTRY: GERMANY: Germany, Federal Republic of
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199710
ENTRY DATE: Entered STN: 19971105
Last Updated on STN: 19971105
Entered Medline: 19971023
AB The centromeres of human chromosomes contain large amounts of the tandemly repeated alpha-satellite DNA family. Previous studies have shown that integration of alpha-satellite DNA into ectopic locations in mammalian chromosomes can result in the de novo formation of several features of centromeric function. Here we further examine the possible centromeric properties of alpha-satellite DNA by introducing it into hamster chromosomes. A large amplified region of ectopic alpha-satellite DNA was shown to direct binding of anticentromere antibodies (ACAs) and centromere protein B (CENP-B). The chromosome containing these ectopic arrays showed a high frequency of formation of anaphase bridges. Owing to the favourable morphology of these chromosomes, we were able to determine that this bridging was due to delayed sister chromatid disjunction at the location of the ectopic alpha-satellite, and not due to de novo formation of a fully functional kinetochore. A separate hamster cell line containing large tandemly repeated amplicons including the DHFR gene also displayed similar behaviour during anaphase. These results may support a role for alpha-satellite DNA in sister chromatid cohesion at centromeres. However, other repetitive DNA in favourable configurations appears to be capable of mimicking this behaviour during anaphase.

L10 ANSWER 53 OF 74 MEDLINE

ACCESSION NUMBER: 97297765 MEDLINE
DOCUMENT NUMBER: 97297765 PubMed ID: 9153397
TITLE: Regulation of serotonin-2C receptor G-protein coupling by RNA editing.
COMMENT: Comment in: Nature. 1997 May 15;387(6630):242-3
AUTHOR: Burns C M; Chu H; Rueter S M; Hutchinson L K; Canton H; Sanders-Bush E; Emeson R B
CORPORATE SOURCE: Department of Pharmacology, Vanderbilt

University School of
Medicine, Nashville, Tennessee 37232-6600, USA.
SOURCE: NATURE, (1997 May 15) 387 (6630) 303-8.
Journal code: NSC; 0410462. ISSN: 0028-0836.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970609
Last Updated on STN: 20000303
Entered Medline: 19970529

AB The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) elicits a wide
array of physiological effects by binding to several receptor
subtypes. The 5-HT2 family of receptors belongs to a large group of
seven-transmembrane-spanning G-protein-coupled receptors and includes
three receptor subtypes (5-HT2A, 5-HT(2B) and 5-HT(2C)) which are linked
to phospholipase C, promoting the hydrolysis of membrane phospholipids and
a subsequent increase in the intracellular levels of inositol phosphates
and diacylglycerol. Here we show that transcripts encoding the 2C subtype
of serotonin receptor (5-HT(2C)R) undergo RNA editing events in which
genomically encoded adenosine residues are converted to inosines by the
action of double-stranded RNA adenosine deaminase(s). Sequence analysis of
complementary DNA isolates from dissected brain regions have indicated
the tissue-specific expression of seven major 5-HT(2C) receptor isoforms
encoded by eleven distinct RNA species. Editing of 5-HT(2C)R messenger
RNAs alters the amino-acid coding potential of the predicted second
intracellular loop of the receptor and can lead to a 10-15-fold reduction
in the efficacy of the interaction between receptors and their G proteins.
These observations indicate that RNA editing is a new mechanism for
regulating serotonergic signal transduction and suggest that this
post-transcriptional modification may be critical for modulating the
different cellular functions that are mediated by other members of the
G-protein-coupled receptor superfamily.

L10 ANSWER 54 OF 74 MEDLINE

ACCESSION NUMBER: 97277029 MEDLINE
DOCUMENT NUMBER: 97277029 PubMed ID: 9130722
TITLE: TRF1 is a dimer and bends telomeric DNA.
AUTHOR: Bianchi A; Smith S; Chong L; Elias P; de Lange T
CORPORATE SOURCE: The Rockefeller University, New York, NY 10021, USA.
SOURCE: EMBO JOURNAL, (1997 Apr 1) 16 (7) 1785-94.
Journal code: EMB; 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199705
ENTRY DATE: Entered STN: 19970609
Last Updated on STN: 19970609
Entered Medline: 19970529

AB TRF1 is a mammalian telomeric protein that binds to the duplex array
of TTAGGG repeats at chromosome ends. TRF1 has homology to the
DNA-binding domain of the Myb family of transcription factors but,
unlike most Myb-related proteins, TRF1 carries one rather than multiple
Myb-type DNA-binding motifs. Here we show that TRF1 binds DNA as a
dimer using a large conserved domain near the N-terminus of the protein
for TRF1-TRF1 interactions. Dimerization was observed both in a complex
with DNA and in the yeast two-hybrid assay. TRF1 dimers were found to
require both Myb repeats for the formation of a stable complex with DNA,
indicating a parallel between the DNA-binding mode of TRF1 and other
Myb-related proteins. TRF1 was found to have a number of biochemical
similarities to Rap1p, a distantly related DNA-binding protein that
functions at telomeres in yeast. Rap1p and TRF1 both require two Myb
motifs for DNA binding and both factors bind along their cognate
telomeric sequences without showing strong cooperative interactions
between adjacent proteins. Furthermore, TRF1 was found to bend its
telomeric site to an angle of -120 degrees. Since Rap1p similarly distorts
telomeric DNA, we propose that DNA bending is important for the
function of telomeres in yeast and mammals.

L10 ANSWER 55 OF 74 MEDLINE

ACCESSION NUMBER: 97146089 MEDLINE
DOCUMENT NUMBER: 97146089 PubMed ID: 8993036
TITLE: Visualization of multicomponent transcription factor complexes on chromatin and nonnucleosomal templates in vivo.
AUTHOR: Archer T K; Lee H L
CORPORATE SOURCE: Department of Obstetrics & Gynaecology, The University of
Western Ontario, London Regional Cancer Centre, Canada.
SOURCE: METHODS, (1997 Feb) 11 (2) 235-45.
Journal code: CPO; 9426302. ISSN: 1046-2023.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199704
ENTRY DATE: Entered STN: 19970414
Last Updated on STN: 19980206
Entered Medline: 19970402

AB There is increasing evidence that specific chromatin structures play an
important role in the regulation of transcription in eukaryotes. The mouse
mammary tumor virus (MMTV) promoter, which is reproducibly assembled into
a phased array of six nucleosomes when introduced into cells, represents
a particularly well-studied example. The second or B nucleosome of the
phased array is disrupted in response to hormone stimulation, allowing
the assembly of a preinitiation complex and the activation of
transcription. In vitro experiments demonstrate that the assembly of the
proximal promoter into chromatin is sufficient to prevent the binding of
transcription factors such as nuclear factor 1. Consequently it is argued
that chromatin serves to restrict the access of ubiquitous transcription
factors in the absence of hormone stimulation. We have employed an in vivo
exonuclease III (ExoIII)/Taq polymerase footprinting assay to study the
hormone-dependent loading of transcription factors on the MMTV promoter.
This assay makes use of stable mouse and human cell lines harboring bovine
papilloma virus chimeras of the MMTV promoter attached to a reporter gene.
To ascertain the significance of protein-chromatin interactions vs
protein-DNA interactions, we examined transcription factor binding to
chromatin and nonchromatin templates of the MMTV promoter within the same
cells. By the use of primers specific for each of the two distinct
reporter genes, and restriction enzymes that generate entry sites for
ExoIII, we can distinguish chromatin and nonnucleosomal templates in vivo.
This system has allowed us to visualize the assembly of multicomponent
transcription preinitiation complexes and to ascertain the consequences of
defined chromatin structures on the binding of individual transcription
factors in vivo.

L10 ANSWER 56 OF 74 MEDLINE

ACCESSION NUMBER: 97112982 MEDLINE
DOCUMENT NUMBER: 97112982 PubMed ID: 8943235
TITLE: The orphan receptor COUP-TF binds to a third glucocorticoid
accessory factor element within the phosphoenolpyruvate carboxykinase gene promoter.
AUTHOR: Scott D K; Mitchell J A; Granner D K
CORPORATE SOURCE: Department of Molecular Physiology and Biophysics,
Vanderbilt University Medical School, Nashville, Tennessee 37232, USA.
CONTRACT NUMBER: DK07061 (NIDDK)
DK20593 (NIDDK)
DK35107 (NIDDK)
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Dec 13) 271 (50)
31909-14.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199701
ENTRY DATE: Entered STN: 19970219
Last Updated on STN: 19990129
Entered Medline: 19970117

AB The phosphoenolpyruvate carboxykinase (PEPCK) gene promoter contains a glucocorticoid response unit (GRU) that includes, as a linear array, two accessory factor binding sites (AF1 and AF2) and two glucocorticoid receptor binding sites. All of these elements are required for a complete glucocorticoid response. AF1 and AF2 also partially account for the response of the PEPCK gene to retinoic acid and insulin, respectively. A second retinoic acid response element was recently located just downstream of the GRU. In this study we show that mutation of the 3' half-site of this element results in a 60% reduction of the glucocorticoid response of PEPCK promoter-chloramphenicol acetyltransferase (CAT) fusion constructs in transient transfection assays, thus the half-site is now termed AF3. A variety of assays were used to show that chicken ovalbumin upstream promoter transcription factor (COUP-TF) binds specifically to AF3 and that upstream stimulatory factor (USF) binds to an E-box motif located 2 base pairs downstream of AF3. Mutations of AF3 that diminish binding of COUP-TF reduce the glucocorticoid response, but mutation of the USF binding site has no effect. The functional roles of AF1, AF2, and AF3 in the glucocorticoid response were explored using constructs that contained combinations of mutations in all three elements. All three elements are required for a maximal glucocorticoid response, and mutation of any two abolish the response.

L10 ANSWER 57 OF 74 MEDLINE

ACCESSION NUMBER: 96347584 MEDLINE
DOCUMENT NUMBER: 96347584 PubMed ID: 8756673
TITLE: Surprising deficiency of CENP-B binding sites in African green monkey alpha-satellite DNA: implications for CENP-B function at centromeres.
AUTHOR: Goldberg I G; Sawhney H; Pluta A F; Warburton P E; Earnshaw W C
CORPORATE SOURCE: Department of Cell Biology and Anatomy, Johns Hopkins School of Medicine, Baltimore, Maryland 21205, USA.
CONTRACT NUMBER: GM35212 (NIGMS)
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1996 Sep) 16 (9) 5156-68.
Journal code: NGY; 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199609
ENTRY DATE: Entered STN: 19961008
Last Updated on STN: 19970203
Entered Medline: 19960926

AB Centromeres of mammalian chromosomes are rich in repetitive DNAs that are packaged into specialized nucleoprotein structures called heterochromatin. In humans, the major centromeric repetitive DNA, alpha-satellite DNA, has been extensively sequenced and shown to contain binding sites for CENP-B, an 80-kDa centromeric autoantigen. The present report reveals that African green monkey (AGM) cells, which contain extensive alpha-satellite arrays at centromeres, appear to lack the well-characterized CENP-B binding site (the CENP-B box). We show that AGM cells express a functional CENP-B homolog that binds to the CENP-B box and is recognized by several independent anti-CENP-B antibodies. However, three independent assays fail to reveal CENP-B binding sites in AGM DNA. Methods used include a gel mobility shift competition assay using purified AGM alpha-satellite, a novel kinetic electrophoretic mobility shift assay competition protocol using bulk genomic DNA, and bulk sequencing of 76 AGM alpha-satellite monomers. Immunofluorescence studies reveal the presence of significant levels of CENP-B antigen dispersed diffusely throughout the nuclei of interphase cells. These experiments reveal a paradox. CENP-B is highly conserved among mammals, yet its DNA binding site is conserved in human and mouse genomes but not in the AGM genome. One interpretation of these findings is that the role of CENP-B may be in the maintenance and/or organization of centromeric satellite DNA arrays rather than a more direct involvement in centromere structure.

L10 ANSWER 58 OF 74 MEDLINE

ACCESSION NUMBER: 96226181 MEDLINE
DOCUMENT NUMBER: 96226181 PubMed ID: 8657576
TITLE: Transcriptional regulation of the MHC class I HLA-A11 promoter by the zinc finger protein ZFX.
AUTHOR: L'Haridon M; Paul P; Xerri J G; Dastot H; Dolliger C; Schmid M; de Angelis N; Grollet L; Sigaux F; Degos L; Gazin C
CORPORATE SOURCE: INSERM U93, Centre Hayem, Paris, France.
SOURCE: NUCLEIC ACIDS RESEARCH, (1996 May 15) 24 (10) 1928-35.
Journal code: O8L; 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960808
Last Updated on STN: 19970203
Entered Medline: 19960731

AB Regulation of the human MHC class I HLA-A11 promoter is governed by a complex array of regulatory elements. One of these elements, shown here to be critical for the transcriptional activity of the promoter, was used to screen a lambda gt11 library and allowed the identification of a cDNA which coded for the zinc finger protein ZFX. ZFX was shown to bind the sequences AGGGCCCCA and AGGCCCCGA, located respectively at positions -271 to -263 and -242 to -234 of the HLA-A11 promoter, with similar affinities through its three C-terminal zinc fingers. ZFX575, a short isoform of ZFX, activates transcription from the HLA-All promoter in a Leydig cell line.

L10 ANSWER 59 OF 74 MEDLINE

ACCESSION NUMBER: 96226164 MEDLINE
DOCUMENT NUMBER: 96226164 PubMed ID: 8657559
TITLE: The viral thymidine kinase gene as a tool for the study of mutagenesis in Trypanosoma brucei.
AUTHOR: Valdes J; Taylor M C; Cross M A; Ligtenberg M J; Rudenko G; Borst P
CORPORATE SOURCE: Division of Molecular Biology, The Netherlands Cancer Institute, Plesmanlaan, Amsterdam.
SOURCE: NUCLEIC ACIDS RESEARCH, (1996 May 15) 24 (10) 1809-15.
Journal code: O8L; 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199607
ENTRY DATE: Entered STN: 19960808
Last Updated on STN: 19960808
Entered Medline: 19960731

AB We have tested the use of thymidine kinase as a negative selection system for Trypanosoma brucei. To this end we have targeted a construct containing a Herpes simplex virus thymidine kinase (TK) gene into the ribosomal DNA array of procyclic T. brucei. This resulted in TK activity 30-50-fold above background and in susceptibility to the nucleoside analogues ganciclovir, ethyl-deoxyuridine and 1-[2-deoxy,2-fluoro-8-D-arabinofuranosyl]-5-iodouracil, all of which have no effect on wild-type trypanosomes. TK+ trypanosomes, however, reverted to a ganciclovir resistant phenotype at a rate of 10(-6) per

cell-generation. A similar reversion rate was observed using the Varicella-zoster virus TK gene. Loss of TK activity was not due to detectable DNA rearrangements or a decrease in TK mRNA. Sequence analysis of the revertant genes demonstrated, however, the occurrence of point mutations and frameshifts. One revertant line had a mutation in the thymidine binding site leading to the substitution of a conserved arginine by a glycine. Other mutations included single base insertion, single base deletion and the introduction of a premature termination codon by point mutation.

L10 ANSWER 60 OF 74 MEDLINE

ACCESSION NUMBER: 96184867 MEDLINE
DOCUMENT NUMBER: 96184867 PubMed ID: 8604351
TITLE: Regulation of ornithine decarboxylase gene expression by the Wilms' tumor suppressor WT1.
AUTHOR: Moshier J A; Skunca M; Wu W; Boppana S M; Rauscher F J 3rd; Dosescu J
CORPORATE SOURCE: Department of Internal Medicine, Wayne State University School of Medicine, Detroit, MI 48201 USA.
CONTRACT NUMBER: CA10815 (NCI) CA47983 (NCI) CA52009 (NCI)
SOURCE: NUCLEIC ACIDS RESEARCH, (1996 Mar 15) 24 (6) 1149-57. Journal code: O8L; 0411011. ISSN: 0305-1048.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199605
ENTRY DATE: Entered STN: 19960524
Last Updated on STN: 20000303
Entered Medline: 19960516

AB The importance of ornithine decarboxylase (ODC) to cell proliferation is underscored by the complex array of cell-specific mechanisms invoked to regulate its synthesis and activity. Misregulation of ODC has severe negative consequences on normal cell function, including the acquisition of tumorigenic growth properties by cells overexpressing ODC. We hypothesize that ODC gene expression is a candidate target for the anti-proliferative function of certain tumor suppressors. Here we show that the Wilms' tumor suppressor WT1 binds to multiple sites within the human ODC promoter, as determined by DNase I protection and methylation interference assays. The expression of WT1 in transfected HCT 116, NIH/3T3 and HepG2 cells represses activity of the ODC promoter controlling expression of a luciferase reporter gene. In contrast WT1 expression enhances ODC promoter activity in SV40-transfected HepG2 cells. Both the extent of modulation of ODC gene expression and the mediating WT1 binding elements are cell specific. Constructs expressing WT1 deletion mutants implicate two regions required for repressor function, as well as an intrinsic activation domain. Understanding the regulation of ODC gene expression by WT1 may provide valuable insights into the roles of both WT1 and ODC in development and tumorigenesis.

L10 ANSWER 61 OF 74 MEDLINE

ACCESSION NUMBER: 96099400 MEDLINE
DOCUMENT NUMBER: 96099400 PubMed ID: 7502076
TITLE: A human telomeric protein.
AUTHOR: Chong L; van Steensel B; Broccoli D; Erdjument-Bromage H; Hanish J; Tempst P; de Lange T
CORPORATE SOURCE: Laboratory for Cell Biology and Genetics, Rockefeller University, New York, NY 10021, USA.
CONTRACT NUMBER: GM49046 (NIGMS) P30 CA08748-29 (NCI)
SOURCE: SCIENCE, (1995 Dec 8) 270 (5242) 1663-7. Journal code: UJ7; 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-U40705
ENTRY MONTH: 199601
ENTRY DATE: Entered STN: 19960217
Last Updated on STN: 19960217
Entered Medline: 19960118

AB Telomeres are multifunctional elements that shield chromosome ends from degradation and end-to-end fusions, prevent activation of DNA damage checkpoints, and modulate the maintenance of telomeric DNA by telomerase. A major protein component of human telomeres has been identified and cloned. This factor, TRF, contains one Myb-type DNA-binding repeat and an amino-terminal acidic domain. Immunofluorescent labeling shows that TRF specifically colocalizes with telomeric DNA in human interphase cells and is located at chromosome ends during metaphase. The presence of TRF along the telomeric TTAGGG repeat array demonstrates that human telomeres form a specialized nucleoprotein complex.

L10 ANSWER 62 OF 74 MEDLINE

ACCESSION NUMBER: 95336660 MEDLINE
DOCUMENT NUMBER: 95336660 PubMed ID: 7612207
TITLE: The use of neuronal networks on multielectrode arrays as biosensors.
AUTHOR: Gross G W; Rhoades B K; Azzazy H M; Wu M C
CORPORATE SOURCE: Department of Biological Sciences, University of North Texas, Denton 76203, USA.
SOURCE: BIOSENSORS AND BIOELECTRONICS, (1995 Summer) 10 (6-7) 553-67. Journal code: AKA; 9001289. ISSN: 0956-5663.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950905
Last Updated on STN: 19990129
Entered Medline: 19950824

AB Mammalian spinal neuronal networks growing on arrays of photoetched electrodes in culture provide a highly stable system for the long-term monitoring of multichannel, spontaneous or evoked electrophysiological activity. In the absence of the homeostatic control mechanisms of the central nervous system, these networks show remarkable sensitivities to minute chemical changes and mimic some of the properties of sensory tissue. These sensitivities could be enhanced by receptor up-regulation and altered by the expression of unique receptors. The fault-tolerant spontaneous network activity is used as a dynamic platform on which large changes in activity signify detection of chemical substances. We present strategies for the expression of novel supersensitivities to foreign molecules via genetic engineering that involves the grafting of ligand binding cDNA onto truncated native receptor DNA and the subsequent expression of such chimeric receptors.

L10 ANSWER 63 OF 74 MEDLINE

ACCESSION NUMBER: 95332316 MEDLINE
DOCUMENT NUMBER: 95332316 PubMed ID: 7608177
TITLE: Characterization of a nuclear protein that interacts with regulatory elements in the human B creatine kinase gene.
AUTHOR: Zhang J N; Wilks J E; Billadello J J
CORPORATE SOURCE: Cardiovascular Division, Washington University School of Medicine, St. Louis, Missouri 63110, USA.
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Jul 7) 270 (27) 16134-9. Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199508
ENTRY DATE: Entered STN: 19950828

Last Updated on STN: 19950828
Entered Medline: 19950816

AB The B creatine kinase gene is regulated by an array of positive and negative cis-elements in the 5'-flanking DNA that function in both muscle and nonmuscle cells. In C2C12 myogenic cells M and B creatine kinase mRNAs are coordinately up-regulated in the early stages of myogenesis and then undergo distinct regulatory programs. The B creatine kinase gene is down-regulated in the late stages of myogenesis as M creatine kinase becomes the predominant species in mature myotubes. Sequences between -92 and +80 of the B creatine kinase gene confer a regulated pattern of expression to chimeric plasmids that closely resembles the time-course of expression of the endogenous B creatine kinase gene in C2C12 cells undergoing differentiation. We show that sequences within the first exon of the B creatine kinase gene are important for the development regulation of the gene in C2C12 cells and that these sequences bind a nuclear protein that shows a similar tissue-specific distribution and developmentally regulated expression to that of the endogenous B creatine kinase gene.

L10 ANSWER 64 OF 74 MEDLINE

ACCESSION NUMBER: 95097999 MEDLINE
DOCUMENT NUMBER: 95097999 PubMed ID: 7799933
TITLE: Mouse mammary tumor virus chromatin in human breast cancer
cells is constitutively hypersensitive and exhibits steroid hormone-independent loading of transcription factors in vivo.
AUTHOR: Mymryk J S; Berard D; Hager G L; Archer T K
CORPORATE SOURCE: Department of Obstetrics & Gynaecology, University of
Western Ontario, London, Canada.
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1995 Jan) 15 (1) 26-34.
Journal code: NGY; 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199501
ENTRY DATE: Entered STN: 19950215
Last Updated on STN: 19970203
Entered Medline: 19950124

AB We have stably introduced a reporter gene under the control of the mouse
mammary tumor virus (MMTV) long terminal repeat (LTR) into human T47D
breast cancer cells to study the action of the progesterone receptor (PR) on transcription from a chromatin template. Unexpectedly, the chromatin organization of the MMTV LTR in these human breast cancer cells differed
markedly from what we have observed previously. The region adjacent to the
transcription start site (-221 to -75) was found to be constitutively hypersensitive to restriction enzyme cleavage in the absence of hormone. This region is normally encompassed within the second nucleosome of a phased array of six nucleosomes that is assembled when the MMTV LTR is
stably maintained in mouse cells. Characteristically, in these rodent cells, the identical DNA sequences show increased restriction enzyme cleavage only in the presence of glucocorticoid. The increased access of restriction enzymes observed in the human PR+ cells was not observed in adjacent nucleosomes and was unaffected by treatment with the progesterone
antagonist RU486. In addition, exonuclease III-dependent stops corresponding to the binding sites for nuclear factor 1 and the PR were observed before and after hormone treatment. These results indicate that MMTV chromatin replicated in these cells is organized into a constitutively open architecture and that this open chromatin state is accompanied by hormone-independent loading of a transcription factor complex that is normally excluded from uninduced chromatin.

L10 ANSWER 65 OF 74 MEDLINE

ACCESSION NUMBER: 95021265 MEDLINE
DOCUMENT NUMBER: 95021265 PubMed ID: 7935450
TITLE: A complex array of positive and negative elements regulates the chicken alpha A-crystallin gene: involvement of Pax-6, USF, CREB and/or CREM, and AP-1 proteins.
AUTHOR: Cvekl A; Sax C M; Bresnick E H; Piatigorsky J
CORPORATE SOURCE: Laboratory of Molecular and Developmental Biology, National
Eye Institute, National Institutes of Health, Bethesda, Maryland 20892-2730.
SOURCE: MOLECULAR AND CELLULAR BIOLOGY, (1994 Nov) 14 (11)
7363-76.
Journal code: NGY; 8109087. ISSN: 0270-7306.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199411
ENTRY DATE: Entered STN: 19941222
Last Updated on STN: 19960129
Entered Medline: 19941118

AB The abundance of crystallins (> 80% of the soluble protein) in the ocular lens provides advantageous markers for selective gene expression during cellular differentiation. Here we show by functional and protein-DNA binding experiments that the chicken alpha A-crystallin gene is regulated by at least five control elements located at sites A (-148 to -139), B (-138 to -132), C (-128 to -101), D (-102 to -93), and E (-56 to -41). Factors interacting with these sites were characterized immunologically and by gel mobility shift experiments. The results are interpreted with the following model. Site A binds USF and is part of a composite element with site B. Site B binds CREB and/or CREM to enhance
expression in the lens and binds an AP-1 complex including CREB, Fra2 and/or JunD which interacts with USF on site A to repress expression in fibroblasts. Sites C and E (which is conserved across species) bind Pax-6 in the lens to stimulate alpha A-crystallin promoter activity. These experiments provide the first direct data that Pax-6 contributes to the lens-specific expression of a crystallin gene. Site D (-104 to -93) binds USF and is a negative element. Thus, the data indicate that USF, CREB and/or CREM (or AP-1 factors), and Pax-6 bind a complex array of
positive and negative cis-acting elements of the chicken alpha A-crystallin gene to control high expression in the lens and repression in fibroblasts.

L10 ANSWER 66 OF 74 MEDLINE

ACCESSION NUMBER: 95011624 MEDLINE
DOCUMENT NUMBER: 95011624 PubMed ID: 7926810
TITLE: Sp1-mediated transactivation of the rabbit alpha 1 acid glycoprotein-encoding gene involves a cis-acting element in the 5'-proximal promoter region.
AUTHOR: Ray B K; Ray A
CORPORATE SOURCE: Department of Veterinary Microbiology, University of
Missouri, Columbia 65211.
SOURCE: GENE, (1994 Sep 30) 147 (2) 253-8.
Journal code: FOP; 7706761. ISSN: 0378-1119.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199411
ENTRY DATE: Entered STN: 19941222
Last Updated on STN: 19941222
Entered Medline: 19941107

AB Analysis of the regulatory promoter region of the rabbit alpha 1-acid glycoprotein (alpha 1-AGP)-encoding gene revealed the presence of a G + C-rich region that is a potential binding site for the transcription factor Sp1. DNase I footprinting and competition with Sp1-specific wild-type oligodeoxyribonucleotides showed that Sp1 interacts with a tandem array of GGGCGG motifs within the alpha 1-AGP promoter. Transfection assays using both liver and nonliver cells have demonstrated that these Sp1-binding elements are transcriptionally active and overproduction of Sp1 can significantly induce the expression of this gene. Previously, we have identified two adjacent C/EBP-binding elements just upstream from these Sp1-binding regions. We now demonstrate by both in vivo and in vitro analyses that C/EBP and Sp1 bind to the alpha 1-AGP promoter and transactivate the expression of this gene in an independent manner.

L10 ANSWER 67 OF 74 MEDLINE

ACCESSION NUMBER: 94235558 MEDLINE
DOCUMENT NUMBER: 94235558 PubMed ID: 8180126
TITLE: Down-regulation of retinoic acid receptor beta in mammary carcinoma cell lines and its up-regulation in senescing normal mammary epithelial cells.
AUTHOR: Swisshelm K; Ryan K; Lee X; Tsou H C; Peacocke M; Sager R
CORPORATE SOURCE: Division of Cancer Genetics, Dana-Farber Cancer Institute, Boston, Massachusetts.
CONTRACT NUMBER: AG-10819 (NIA) CA-39814 (NCI) T32 CA-09361 (NCI) +
SOURCE: CELL GROWTH AND DIFFERENTIATION, (1994 Feb) 5 (2) 133-41. Journal code: AYH; 9100024. ISSN: 1044-9523.
PUB. COUNTRY: United States Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199406
ENTRY DATE: Entered STN: 19940621 Last Updated on STN: 19980206 Entered Medline: 19940614
AB Retinoids are important cellular, dietary factors that regulate differentiation and cellular growth. They serve as ligands for specific nuclear receptors, the retinoic acid receptors (RARs). Ligand-activated receptors regulate gene transcription through target retinoic acid-responsive elements (RAREs) found in promoter regions. We have investigated the expression of retinoic acid receptor genes (alpha, beta, gamma) and retinoid X receptor beta in normal, senescing, and tumorigenic human mammary epithelial cells. We find that most tumor cells show a loss of RAR-beta expression, but that RAR-alpha and -gamma as well as retinoid X receptor beta are variably expressed in both normal and tumor cells. RAR-beta gene expression is induced both by retinoic acid and by fenretinide in normal cells, but tumor cells fail to respond to either. In contrast, RAR-beta expression increases with serial passage in senescing cells. Paradoxically, both normal and tumor cells can trans-activate an exogenous beta-RARE, as demonstrated by reporter gene assays. Oligonucleotide mobility shift assays with the beta-RARE show a single discrete complex in normal cells, whereas tumor cells exhibit a heterogeneous set of larger complexes, which indicates that tumor cells utilize a different array of factors within the beta-RARE. Reporter gene assays with extended promoter regions indicate the presence of negative regulatory elements and/or factor binding sites that reside between -1500 and the RARE located at -59, and that the promoter is down-regulated in MCF-7 tumor cells. Our findings reveal a dichotomy: RAR-beta transcription is down-regulated in tumor cells compared with normal human mammary epithelial cells, and up-regulated in senescence.

L10 ANSWER 68 OF 74 MEDLINE

ACCESSION NUMBER: 94068381 MEDLINE
DOCUMENT NUMBER: 94068381 PubMed ID: 8248099
TITLE: Construction of multiple copy of alpha-domain gene fragment of human liver metallothionein IA in tandem arrays and its expression in transgenic tobacco plants.
AUTHOR: Pan A; Tie F; Yang M; Luo J; Wang Z; Ding X; Li L; Chen Z; Ru B
CORPORATE SOURCE: National Laboratory of Protein Engineering and Plant Genetic Engineering, Department of Biology, Peking University, Beijing, China.
SOURCE: PROTEIN ENGINEERING, (1993 Sep) 6 (7) 755-62. Journal code: PR1; 8801484. ISSN: 0269-2139.
PUB. COUNTRY: ENGLAND: United Kingdom Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199401
ENTRY DATE: Entered STN: 19940201 Last Updated on STN: 19970203 Entered Medline: 19940106
AB Metallothioneins (MT) are low molecular weight, cysteine-rich, metal-binding proteins. An MT molecule contains two domains which appear to act independently--an alpha-domain, which is characterized by cadmium-binding, and a beta-domain, which binds preferentially to copper. Based on this conception, DNA duplex encoding the alpha-domain (106 bp) of human MT-IA was constructed from a chemically-synthesized oligomer by repair synthesis and enzymatic ligation and cloned into pUC19. The genes cloned were sequenced and found to be in the correct order as designed. Synthetic directional adapters were attached to the terminals of the alpha-domain gene fragment of human MT-IA to establish complete control over fragment orientation during ligation. The use of these directional adapters thereby ensured the production of multiple copies of the alpha-domain in tandem arrays. The successive alpha-domains were linked by a peptide linker consisting of 10 residues. A chimeric gene containing 12 cloned tandemly repeated copies of the 106 bp alpha-domain DNA was introduced into tobacco cells on a disarmed Ti-plasmid of Agrobacterium tumefaciens. A total of 10 different transgenic tobacco plants were generated, of which two showed root and shoot growth unaffected by up to 200 mg/l kanamycin and 100 microM cadmium, whereas root growth of control plants was severely inhibited and leaf chlorosis developed on media containing only 10 microM cadmium.

L10 ANSWER 69 OF 74 MEDLINE

ACCESSION NUMBER: 93081789 MEDLINE
DOCUMENT NUMBER: 93081789 PubMed ID: 1450410
TITLE: T-cell acute lymphoblastic leukemia--the associated gene SCL/tal codes for a 42-Kd nuclear phosphoprotein.
AUTHOR: Goldfarb A N; Goueli S; Mickelson D; Greenberg J M
CORPORATE SOURCE: University of Minnesota Hospital Department of Laboratory Medicine and Pathology, Minneapolis.
CONTRACT NUMBER: CA-01254 (NCI)
SOURCE: BLOOD, (1992 Dec 1) 80 (11) 2858-66. Journal code: A8G; 7603509. ISSN: 0006-4971.
PUB. COUNTRY: United States Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals
ENTRY MONTH: 199212
ENTRY DATE: Entered STN: 19930129 Last Updated on STN: 19970203 Entered Medline: 19921231
AB SCL/tal is a putative oncogene originally identified through its involvement in the translocation t(1;14)(p32;q11) present in the leukemic cell line DU.528. Subsequent studies have shown an upstream deletion activating expression of SCL/tal to be one of the most common genetic lesions in T-cell acute lymphoblastic leukemia (T-ALL). The cDNA sequence of SCL/tal encodes a basic helix-loop-helix (bHLH) protein with regions of marked homology to lyl-1 and tal-2, two other bHLH proteins involved in T-ALL chromosomal translocations. The bHLH motif suggests that the SCL/tal product localizes to the nucleus, binds to specific DNA sequences, and regulates transcription of a specific array of target genes. Our studies directly identify the SCL/tal product as a 42-Kd phosphoprotein that efficiently localizes to the nucleus. Deletion mutagenesis has allowed identification of a region critical for nuclear localization, a region that corresponds to the DNA-binding basic domain within the bHLH motif. Because this domain is shared by lyl-1 and tal-2, these latter putative T-cell oncoproteins probably use a nuclear localization mechanism identical to that of SCL/tal.

L10 ANSWER 70 OF 74 MEDLINE

ACCESSION NUMBER: 92380993 MEDLINE

DOCUMENT NUMBER: 92380993 PubMed ID: 1512232
TITLE: Localization of O-GlcNAc modification on the serum response
transcription factor.
AUTHOR: Reason A J; Morris H R; Panico M; Marais R; Treisman R H;
Haltiwanger R S; Hart G W; Kelly W G; Dell A
CORPORATE SOURCE: Department of Biochemistry, Imperial College of Science,
Technology, and Medicine, London, United Kingdom.
CONTRACT NUMBER: CA 42486 (NCI)
HD 13563 (NICHD)
+
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1992 Aug 25) 267 (24)
16911-21.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199209
ENTRY DATE: Entered STN: 19921018
Last Updated on STN: 19921018
Entered Medline: 19920925

AB A unique form of nucleoplasmic and cytoplasmic protein glycosylation, O-linked GlcNAc, has previously been detected, using Gal transferase labeling techniques, on a myriad of proteins (for review see Hart, G. W., Haltiwanger, R. S., Holt, G. D., and Kelly, W. G. (1989a) Annu. Rev. Biochem. 58, 841-874), including many RNA polymerase II transcription factors (Jackson, S. P., and Tjian, R. (1988) Cell 55, 125-133). However, virtually nothing is known about the degree of glycosylation at individual sites, or, indeed, the actual sites of attachment of O-GlcNAc on transcription factors. In this paper we provide rigorous evidence for the occurrence and locations of O-GlcNAc on the c-fos transcription factor, serum response factor (SRF), expressed in an insect cell line. Fast atom bombardment mass spectrometry (FAB-MS) of proteolytic digests of SRF provides evidence for the presence of a single substoichiometric O-GlcNAc residue on each of four peptides isolated after sequential cyanogen bromide, tryptic, and proline specific enzyme digestion: these peptides are 306VSASVSP312, 274GTTSTIQTAP283, 313SAVSSADGTVLK324, and 374DSSTDLTQTSSSGTVTLP391. Using an array of techniques, including manual Edman degradation, aminopeptidase, and elastase digestion, together with FAB-MS, the major sites of O-GlcNAc attachment were shown to be serine residues within short tandem repeat regions. The highest level of glycosylation was found on the SSS tandem repeat of peptide (374-391) which is situated within the transcriptional activation domain of SRF. The other glycosylation sites observed in SRF are located in the region of the protein between the DNA binding domain and the transcriptional activation domain. Glycosylation of peptides (274-283) and (313-324) was found to occur on the serine in the TTST tandem repeat and on serine 316 in the SS repeat, respectively. The lowest level of glycosylation was recovered in peptide (306-312) which lacks tandem repeats. All the glycosylation sites identified in SRF are situated in a relatively short region of the primary sequence close to or within the transcriptional activation domain which is distant from the major sites of phosphorylation catalyzed by casein kinase II.

L10 ANSWER 71 OF 74 MEDLINE

ACCESSION NUMBER: 92261605 MEDLINE
DOCUMENT NUMBER: 92261605 PubMed ID: 1584220
TITLE: Capacity for cooperative binding of thyroid hormone (T3) receptor dimers defines wild type T3 response elements.
AUTHOR: Brent G A; Williams G R; Harney J W; Forman B M; Samuels H
H; Moore D D; Larsen P R
CORPORATE SOURCE: Thyroid Division, Brigham and Women's Hospital, Boston,
Massachusetts 02115.
CONTRACT NUMBER: 5-K12-AM01401 (NIADDK)
DK-16636 (NIDDK)
DK-36256 (NIDDK)
SOURCE: MOLECULAR ENDOCRINOLOGY, (1992 Apr) 6 (4) 502-14.
Journal code: NGZ; 8801431. ISSN: 0888-8809.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199206
ENTRY DATE: Entered STN: 19920626
Last Updated on STN: 19970203
Entered Medline: 19920612

AB Thyroid hormone response elements (T3REs) have been identified in a variety of promoters including those directing expression of rat GH (rGH), alpha-myosin heavy chain (rMHC), and malic enzyme (rME). A detailed biochemical and genetic analysis of the rGH element has shown that it consists of three hexamers related to the consensus [(A/G)GGT(C/A)A]. We have extended this analysis to the rMHC and rME elements. Binding of highly purified thyroid hormone receptor (T3R) to T3REs was determined using the gel shift assay, and thyroid hormone (T3) induction was measured in transient tranfections. We show that the wild type version of each of the three elements binds T3R dimers cooperatively. Mutational analysis of the rMHC and rME elements identified domains important for binding T3R dimers and allowed a direct determination of the relationship between T3R binding and function. In each element two hexamers are required for dimer binding, and mutations that interfere with dimer formation significantly reduce T3 induction. Similar to the rGH element, the rMHC T3RE contains three hexameric domains arranged as a direct repeat followed by an inverted copy, although the third domain is weaker than in rGH. All three are required for full function and T3R binding. The rME T3RE is a two-hexamer direct repeat T3RE, which also binds T3R monomer and dimer.
Across a series of mutant elements, there was a strong correlation between dimer binding in vitro and function in vivo for rMHC (r = 0.99, P less than 0.01) and rME (r = 0.67, P less than 0.05) T3REs. Our results demonstrate a similar pattern of T3R dimer binding to a diverse array of hexameric sequences and arrangements in three wild type T3REs. Addition of nuclear protein enhanced T3R binding but did not alter the specificity of binding to wild type or mutant elements. Binding of purified T3R to T3REs was highly correlated with function, both with and without the addition of nuclear protein. T3R dimer formation is the common feature which defines the capacity of these elements to confer T3 induction.

L10 ANSWER 72 OF 74 MEDLINE

ACCESSION NUMBER: 92196588 MEDLINE
DOCUMENT NUMBER: 92196588 PubMed ID: 1347958
TITLE: Transcription factor loading on the MMTV promoter: a bimodal mechanism for promoter activation.
COMMENT: Erratum in: Science 1992 Apr 10;256(5054):161
AUTHOR: Archer T K; Lefebvre P; Wolford R G; Hager G L
CORPORATE SOURCE: Hormone Action and Oncogenesis Section, Laboratory of
Molecular Virology, National Cancer Institute, Bethesda, MD 20892.
SOURCE: SCIENCE, (1992 Mar 20) 255 (5051) 1573-6.
Journal code: UJ7; 0404511. ISSN: 0036-8075.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199204
ENTRY DATE: Entered STN: 19920509
Last Updated on STN: 19970203
Entered Medline: 19920423

AB The mouse mammary tumor virus (MMTV) promoter attains a phased array of six nucleosomes when introduced into rodent cells. This architecture excludes nuclear factor 1/CCAAT transcription factor (NF1/CTF) from the promoter before glucocorticoid treatment and hormone-dependent access of nucleolytic agents to promoter DNA. In contrast, when the promoter was

transiently introduced into cells, NF1/CTF was bound constitutively and nucleolytic attack was hormone-independent. Thus, induction at this promoter was a bimodal process involving receptor-dependent remodeling of chromatin that allows NF1/CTF loading and direct receptor-mediated recruitment of additional transcription factors.

L10 ANSWER 73 OF 74 MEDLINE

ACCESSION NUMBER: 92007746 MEDLINE
DOCUMENT NUMBER: 92007746 PubMed ID: 1915276
TITLE: A negatively acting DNA sequence element mediates phytochrome-directed repression of phyA gene transcription.
AUTHOR: Bruce W B; Deng X W; Quail P H
CORPORATE SOURCE: University of California, Berkeley/US Department of Agriculture, Plant Gene Expression Center, Albany 94710.
SOURCE: EMBO JOURNAL, (1991 Oct) 10 (10) 3015-24.
Journal code: EMB; 8208664. ISSN: 0261-4189.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199110
ENTRY DATE: Entered STN: 19920124
Last Updated on STN: 19980206
Entered Medline: 19911025

AB Phytochrome represses transcription of its own phyA genes within 5 min of light-triggered conversion to its active Pfr form. We have utilized microprojectile mediated gene transfer into etiolated rice seedlings to delineate sequence elements in the oat phyA3 promoter responsible for this regulation. Linker-scan mutagenesis of this promoter has identified two positive elements which together are necessary for maximal transcription in the absence of Pfr. These elements are designated PE1, centered at position -357 bp, and PE3, centered at position -96 bp. Sequence mutagenesis immediately downstream of PE3 results in maximal transcription in the presence of high Pfr levels, indicating that Pfr represses phyA3 transcription through a negatively acting sequence element. This element, designated RE1, with the sequence CATGGGCGCGG, encompasses a motif that is highly conserved in all monocot phyA promoters thus far characterized. DNase I protection analysis indicates that oat nuclear extracts contain multiple factors that bind to an array of sequence motifs, including PE1 and part of PE3, within 400 bp upstream of the oat phyA3 transcription start site. This DNA-binding pattern is not altered by Pfr. Weak binding to part of the RE1 motif is evident but also with no difference between high and low Pfr levels. We conclude that the signal transduction chain that mediates Pfr-directed repression of phyA3 transcription terminates with a negatively acting transcription factor that binds to the sequence element RE1.

L10 ANSWER 74 OF 74 MEDLINE

ACCESSION NUMBER: 91155957 MEDLINE
DOCUMENT NUMBER: 91155957 PubMed ID: 1963474
TITLE: An interaction between the 5' flanking distal and proximal regulatory domains of the rat prolactin gene is required for transcriptional activation by estrogens.
AUTHOR: Seyfred M A; Gorski J
CORPORATE SOURCE: Department of Molecular Biology, Vanderbilt University, Nashville, Tennessee 37235.
CONTRACT NUMBER: CA-18110 (NCI)
HD-07259 (NICHD)
HD-0819 (NICHD)
+
SOURCE: MOLECULAR ENDOCRINOLOGY, (1990 Aug) 4 (8) 1226-34.
Journal code: NGZ; 8801431. ISSN: 0888-8809.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199104
ENTRY DATE: Entered STN: 19910428
Last Updated on STN: 19910428
Entered Medline: 19910409

AB In vitro studies have demonstrated that the estrogen receptor (ER) can bind to the rat PRL estrogen response element (ERE) located 1700 basepairs upstream of the transcriptional start site. However, the mechanism by which the receptor-DNA complex influences the activity of RNA polymerase located in the promoter region is not understood. To begin investigating this process, we developed cell lines derived from GH3 cells that contain steroid-responsive bovine papillomavirus minichromosomes. Within these minichromosomes is a hybrid gene composed of the 5' flanking region of the PRL gene, driving the expression of the Tn5 gene. The episomal PRL DNA sequences responded to 17 beta-estradiol (E2) by increasing the rate of Tn5 gene transcription. Nucleosome mapping experiments using micrococcal nuclease demonstrated that nucleosome-like structures were assembled on the minichromosome in an ordered array separated by 150-200 basepairs of DNA. Novel S1 nuclease as well as DNase-I-hypersensitive sites in the chromatin of the promoter and distal regulatory regions of the episomal PRL gene were detected by indirect end-labeling studies. The nuclease hypersensitive sites in the distal region containing the ERE were modified after treatment of the cells with either E2 or the antiestrogen 4-hydroxytamoxifen. However, only E2 treatment of cells resulted in an increase in the nuclease hypersensitivity of the promoter region and induced gene expression, while antiestrogen treatment had no effect on either parameter. This suggests that complex interactions between factors located at the distal and proximal regulatory regions ultimately determine the transcriptional response of the PRL gene to E2.